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A RAPID CELLOIDIN METHOD FOR THE ROTARY MICROTOME

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ABSTRACT.—**A method is described which combines the writer's hot celloidin technic[1] with a form of the clearing-before-cutting procedure. The method requires only 16–17 days and yields a block which may be cut in any microtome, the sections being as thin as those afforded by paraffin with comparable material. The advantages of celloidin over paraffin, listed in the writer's earlier paper, are retained in the present method which, altho consuming more time than the hot process, requires less skill and gives superior results.**

In an earlier paper,[1] which must be consulted by the reader of the present one, the writer described a rapid celloidin method, the central feature of which is the elimination of evaporation as a means of increasing the concentration of the celloidin during infiltration and inclusion. In the classical cold celloidin method, evaporation of the dual solvent is permitted to start early in the procedure and, as the ether escapes faster than the alcohol, the result is a matrix which apparently "sets" largely by precipitation from a no longer adequate solvent, yielding a block whose actual celloidin content is low, and which is too milky and rubbery to permit easy orientation and thin sectioning.

The "hot" method involves a forced increase in concentration by frequent changes of the solution, and under its conditions the concentration can be carried much higher before solidification is permitted. While the hot method is very rapid, it has two disadvantages: a sliding microtome must be used, and considerable skill is needed in the cutting process if the most is to be gained from the method in the way of thin, perfect sections.

The technic here described combines the rapid infiltration of the hot process with a different method of inclusion, in which carefully controlled evaporation and impregnation with cedar oil are employed.

[1]Walls, G. L. The hot celloidin technic for animal tissues. Stain Techn., **7**, 135–145. 1932.

The latter step will be familiar, as it is the essential departure of the clearing-before-cutting modification of the ordinary cold method. It permits dry storage and dry cutting in a rotary microtome—which should make the present method appeal to those who have only a rotary machine available and wish to be able to secure their occasional celloidin blocks in a reasonable time. The writer would mention, however, that he personally employs this method not as an occasional last-resort substitute for paraffin, but routinely in decided preference to the latter matrix for any sort of material.

Procedure

Secure the materials, and prepare the solutions listed on pp. 136–138 of the writer's earlier paper, substituting 15% celloidin for the 14% solution and omitting the 50–50 mixture of alcohol and glycerin. In place of the latter, some Gilson's mixture (50–50 chloroform and cedar oil) and some cedar oil will be needed. The latter should be the kind marked "water-white" or "microscopic, for clearing"—*not* the thick kind used for immersion optics. A few short style ½-pint clamp-top Mason jars (or similar fruit-preserving jars), with a 100 cc. beaker for each, will be required.

Follow the hot process, using 2%, 4%, 6%, 10%, and 15% solutions for 24 hours each[2]; but add no dry celloidin with the thickest solution as in the straight hot method. After 24 hours in 15% celloidin in the incubator, transfer the pieces of tissue to about 60 cc. of 15% in a beaker, place the latter in one of the Mason jars, and clamp the top on the jar *leaving off the rubber sealing-ring.*

Slow evaporation will now begin, and in about four days the mass will be deeply concave on top. Run a dissecting needle around the top of the mass to free it from the glass, and replace the top on the jar (if this results in the formation of bubbles at the bottom of the beaker, wait longer the next time). Weather conditions will govern the duration of the inclusion process, but after a total of five to eight days in the beaker the mass should be capable of being shaken out into the hand. It may be necessary to pull the mass out with forceps and replace it a day or so before this stage is reached in order that it will free itself naturally from the beaker and not remain stuck to the latter until it has become *too* hard.

If the mass feels dry, is fairly hard to squeeze, and does not show light fingerprints readily, it is ready to block: trim (not too closely; dry all trimmings, to re-dissolve for later use) and cement to a fiber

[2]It is best to invert the embedding bottles in the incubator to seal the corks well. This point was inadvertently omitted from the description of the hot method.

microtome block[3] with 15% celloidin, first dipping the surfaces to be apposed in ether-alcohol. Place in Gilson's mixture for 24 hours, then blot the block and allow it to stand in air for ten to twenty minutes, depending upon size. Transfer to cedar oil. Blocks must remain here for at least three days before cutting, and may be stored indefinitely in cedar oil either before or after sections have been cut from them. After a month or so they may even be stored dry in an air-tight jar.

To section, wipe the block dry and cut in either a rotary or a heavy sliding microtome, with the knife in standard paraffin position—not slanted as for wet celloidin blocks. Cut rapidly and transfer sections in batches of ten or more, with brush or forceps, to 80% alcohol.

Rinse the sections in 95% alcohol to remove the cedar oil, and transfer to clean 80%. They can stay here indefinitely. They will, in fact, be toughened somewhat by the 80% in a week or so.

When ready to stain, transfer directly to water (adding a *trace* of soap to the water if the "scooting-about" phenomenon promises damage to very delicate sections) and follow the procedure outlined on pp. 141–142 of the paper on the hot process.

Remarks

This "hot-dry" method is not entirely foolproof, as some judgment is required in determining the stopping point of the evaporation. The use of the fruit jar and beaker, however, slows the latter process to such a degree that both of the solvent liquids are able to escape properly, so that the matrix remains transparent and is high in final celloidin content. The finished block is very hard and can usually be cut at 6μ, often thinner.

The evaporation can be stopped at any time, if one has not time to block the matrix when it is ready, simply by putting the sealing-ring in place on the clamped jar.

There are two difficulties which may arise:

1) When flat pieces of tissue, such as patches of skin, etc., are to be embedded, they must not be allowed to lie on the bottom of the beaker, else there will be no celloidin to speak of on one side of them when blocked. The writer has tried various metal props, which were pulled out of the mass at the time of blocking, but has discarded them in favor of the following procedures, between which there is little to choose—one involving a little more trouble, the other a little more time: *a*) When the mass is at blocking consistency, trim the concave top to a plane with a knife, invert the mass in the beaker,

[3]Which is labeled with India ink on its under side.

and add a little more 15% celloidin. Replace in the Mason jar, and in a day or two the mass will regain homogeneity. There will now be a safe thickness of celloidin on the formerly exposed side of the tissue. *b*) When doing routine blocking, cut discs from the mass before trimming out the cylinder containing the tissue and store these in an air-tight jar. Place one of the discs in the bottom of the beaker whenever starting a flat piece of tissue thru the evaporation process.

2) Bubbles may form in the beaker (especially in warm weather) and, if large and in contact with the tissue, may give difficulty in sectioning. Bubble formation is an indication that evaporation is taking place too rapidly, or else that the mass has been freed from the sides of the beaker too soon. If the bubbles appear while the celloidin is still quite fluid, they may be caused to shrink and vanish by putting the sealing-ring on the Mason jar and placing the latter in a refrigerator for a few hours (allowing the jar to regain room temperature before opening it and removing the sealing-ring). If they form after the mass has become semi-solid, the only remedy is to fill those which are in dangerous locations at the time of blocking with 15% celloidin using a hypodermic syringe.

MICROTOME KNIFE SHARPENERS OPERATING ON THE ABRASIVE-GROUND GLASS PRINCIPLE[1]

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ABSTRACT.—**Developments in the abrasive-ground glass type of microtome knife sharpener have been traced from 1857 to the present. Merits and disadvantages of each apparatus are critically discussed, particularly of the two practical power sharpeners, namely, the horizontal revolving disc and the vertical wheel. A new vertical wheel model, designed to facilitate the sharpening of long, sliding microtome knives, either with a chisel-shaped or a symmetrical bevel, is described. Notable mechanical features include: shaft rotating on V-slots as sole adjustable part, thereby assuring accuracy of alignment; steel guide bar 45.7 cm. long; glass wheel mounted on its own axis, separate from motor; simple and sturdy construction with single casting constituting entire framework.**

HISTORICAL

Altho second in importance only to the microscope itself, the earliest "microtome" was simply a knife or razor. Yet so proficient were early microscopists in its use that as late as fifty years ago the finest and most critical sections were cut free hand. Little wonder, then, that the condition of the knife was a matter for primary consideration and that no pains were spared in order to produce a perfect cutting edge. That they succeeded to a remarkable degree is shown by the fact that the best sharpening methods known today are fundamentally identical with those of eighty years ago. It seems proper, therefore, to preface a discussion of modern microtome knife sharpeners with a sketch of their predecessors.

Realizing that it was rarely possible to have microtome knives well sharpened by another, Hugo von Mohl (1857) developed a technic of his own, which, he predicted, anyone with a little mechanical skill could soon acquire. His principal innovation was the use of a thick plate glass having a plane and very finely ground surface, upon which the previously honed knife was polished. After adding to the plate a creamy paste of a fine abrasive powder, usually Vienna chalk, a circular motion was imparted to the knife, it being considered unessential for polishing that the knife always move against its edge as in honing. The blade was turned over at frequent intervals and progress

[1]Apparatus constructed for an investigation supported by a grant from The Rockefeller Foundation to Prof. T. H. Goodspeed.

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was ascertained by repeated inspections under the microscope. This method was apparently widely adopted and was favored by Harting (1866), Dippel (1885), Moll (1892), Bouin and others. One reason for this popularity, mentioned by Harting (1866), was that good hones were expensive and soon became concave, whereas one glass plate could be readily discarded for another. It thus simplified the problem of maintaining a knife with a straight edge and a straight bevel. Twenty-seven years after its introduction, Dippel (1885) wrote, "For polishing, the method recommended by Hugo von Mohl is unsurpassed."

Naturally this initial success led to attempts to improve upon both the form and usefulness of the original apparatus. The glass soon came to be used as a base for the coarser grinding as well as for the polishing, and later Lendvai (1909) advocated a set of three plates, one for each grade of abrasive powder. The knives were held either flat on the plate or against tubular steel backs with which they were equipped, but the glass plates were still similar in size to the regular hones and continued to be used in the same manner. In a recent apparatus designated as the "Schmid Sharpener" (A. H. Thomas Co., Philadelphia) the knife holder consists of a carriage mounted on wheels and a rod which screws into the back of the knife. A smooth glass plate serves as a runway for the holder while the knife is alternately pushed and pulled across a ground glass surface large enough (35 x 35 cm.) to accommodate its full length. Altho this constituted a definite advance, the sharpening process still remained a task both laborious and time consuming.

The application of power to the problem of microtome knife sharpening has resulted in the development of three types of apparatus. For brevity, these may be referred to as the (1) sliding plate, (2) horizontal revolving disc, and (3) vertical wheel types. The sliding plate mechanism was built by Funck (1910), who first used water power and later an electric motor. Sliding back and forth three times a second on a 14 cm. track, the glass plate had an average linear velocity of 50 meters per minute. The knife was held against the plate by hand, either perpendicularly or obliquely to the line of motion, during both forward and backward strokes. Various grades of levigated alumina were employed to form abrasive pastes. Inherently impractical for a sharpener from the mechanical standpoint, it is improbable that this type of construction will ever be widely used. Funck himself seems not to have been satisfied with it, for he also designed a model with a horizontal revolving disc (200 r. p. m.). He did not regard the higher speed of the latter as an advantage, however, and was deterred from executing his plans

by the disadvantage of rapid loss of abrasive, possible accidental injury, and the unequal grinding of the knife along its length. Only the last-mentioned disadvantage possesses fundamental significance.

A contemporary, Stefan von Apathy (1913), who has recorded his dissatisfaction with knives sharpened by Funck, was using a horizontal disc at about the same time, but has supplied no further details. An apparatus of this type developed by Nageotte (1926) had a disc 43 cm. in diameter with a 20 cm. hole at the center, thus furnishing an effective surface 11.5 cm. wide. It revolved once a second (60 r. p. m.). Nageotte found a principal difficulty in constructing satisfactory knife holders and his final choice, one which fitted over the back of the knife and rested on the glass disc during sharpening, was not a desirable solution of the problem. Besides being ground away as rapidly as the knife edge, the holder required the polishing to be done at the same angle as the grinding.

For both the sliding plate and horizontal disc sharpeners, a mechanical method of supporting the knife seems best suited. Fanz (1929) has added this improvement to his automatic horizontal plate sharpener, in which the knife slowly oscillates back and forth, and periodically reverses from one side to the other. This machine is offered commercially in two sizes, 45 cm. and 75 cm. disc, with the automatic features optional. It operates at a speed of 42 revolutions per minute. Since the tangential velocity of a point on the disc increases as its distance from the center, it follows that the abrasive action on a knife is a linear function of its radial position. Simultaneously, two problems are created. The first is how to compensate for the unequal grinding of the knife along its length in order to maintain a straight edge parallel to the back. This can be done by reversing the direction of rotation of the disc during half of the sharpening process, but this may prove very difficult to do with automatic machines. It may not be necessary for rotary microtome knives. The second and more serious problem is how to maintain the ground glass surface of the disc plane. If facilities are not available for resurfacing the disc, probably the simplest and most economical, altho but partial, solution is to replace it occasionally with a new one.

Vertical Wheel Sharpeners

The vertical wheel microtome knife sharpener designed by Prof. J. A. Long (1927) of this institution, departs radically from the flat plate characteristics of all previous apparatus based on the abrasive-ground glass principle. In this case, the periphery of a disc rotating in a vertical plane forms the grinding surface. The knife edge is passed laterally across this surface by an operator who holds the

back of the knife firmly against the two sides of an L-shaped guide bar (Fig. 1). Since no auxiliary back or holder is required, a possible source of inaccuracy is eliminated and the mechanical stability increased. It is only essential that the edges of the back of the knife be straight and parallel to the cutting edge. In case the narrow (1.3 cm.) wheel should wear smooth or become uneven, it can be simply redressed. The wheel housing acts as a reservoir for the abrasive suspension, which the wheel itself automatically circulates. The concavity produced in a narrow bevel by polishing on a 15 cm.

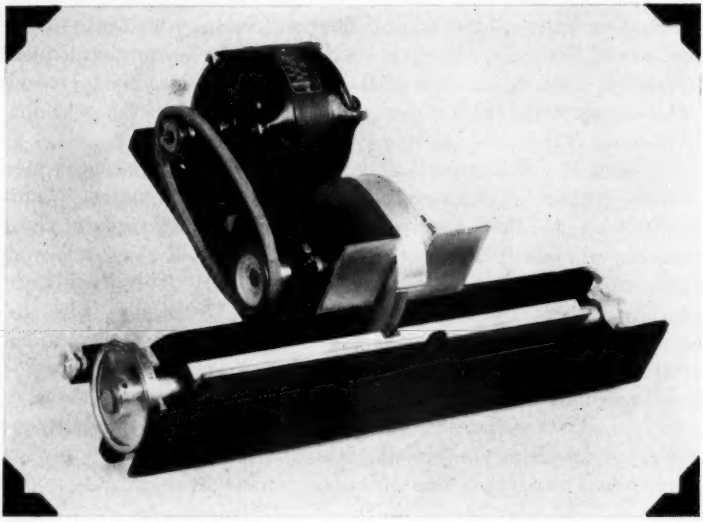

Fig. 1. A microtome knife sharpener employing a vertical glass wheel with a finely ground surface. An operator slides the knife back and forth across the narrow face of the wheel, at the same time holding the back of the knife firmly against the long L-shaped guide bar. See text for further details.

wheel is so slight as to be of no practical consequence. It will be seen that the objections cited by Funck to the horizontal disc do not apply to the vertical wheel provided the operator imparts a uniform lateral velocity to the knife. When this relatively simple skill is once acquired, the bevel can be abraded uniformly along its length, as demanded for sliding microtome knives. Stropping is unnecessary and it is best to dispense with it entirely. Polishing is normally done without any abrasive save the ground glass surface itself, with distilled water, dilute alcohol, or other liquid to prevent heating (cf. Garland, 1935). The operating speed may be high (1800 r. p. m.

for a 15 cm. wheel) provided the knife is moved rapidly enough across the wheel and the pressure against the knife is not too great.

The vertical wheel sharpener shown in Figure 1 was designed by the writer to accommodate the longer knives used on sliding microtomes and to facilitate the reconditioning of knives possessing a chisel shaped bevel. It differs from the Long model just described in size and in its general mechanical execution. The aim has been to attain the highest degree of accuracy and manipulative convenience compatible with simplicity and sturdiness of construction. The details of the mechanical design, many of which will be sufficiently clear from the accompanying photograph, will be concisely described.

A single brass casting weighing 23 kg. constitutes the entire framework for the apparatus including the base for the motor, the lower housing of the glass wheel, and the long trough (53 cm.) containing the guide bar assembly. In order that the abrasive suspension may flow back into its reservoir under the glass wheel, the bottom of the trough slopes down slightly from the ends to the center and the forward part of the apparatus stands on two legs, 5 cm. high. A stopcock on the bottom of the reservoir enables it to be emptied conveniently. The housing over the wheel is so fashioned as to direct an abundant supply of fluid to the point of contact between wheel and knife. To prevent possible splashing in the direction of the operator, an appropriate vertical projection has been designed on the front wall of the casting. The manner in which the steel guide bar unit has been assembled can be seen from the photograph. The bars of square (1.9 x 1.9 cm.) and rectangular (0.63 x 5.08 cm.) cross section are 45.7 cm. long, whereas the round cold rolled steel shafting is 2.54 cm. in diameter and 61 cm. long. The square bar has been recessed in the center to allow the closer approach required for small knives and to prevent splashing. Several small holes in the rectangular bar opposite the wheel permit a rapid drainage of the grinding fluid. To prevent rusting, all the steel parts have been "hard" chromium plated directly onto the steel in a hot solution, but how long such plating will resist abrasive action effectively is not yet known. The steel shaft is supported near the ends on V-slots in the brass casting, wherein it can be rigidly clamped by a partial turn of the thumb screw in the top thrust shown at the right end. Since the rotation of this shaft constitutes the sole operating adjustment of the sharpener, its accuracy of alignment is imperative. This accuracy can be attained in no better or simpler way than by the use of a round shaft in widely separated V-slots. A fine adjustment is furnished by means of a pinion and spur gear visible at the left end.

The positions of the guide bar are read on the circular scale and vernier mounted at the left end of the shaft. The circular scale consists of a flexible steel metric rule fastened on the periphery of a 10 cm. disc. Since bevel angles cannot be read directly in degrees where knives are of various dimensions, it is advisable to prepare a calibration chart for each size knife so that the bevel angles may be known.

In order to produce a chisel type knife edge or one having unsymmetrical bevels, the guide bar must be set alternately at either of two positions, corresponding to the two bevel angles desired, whenever the knife is reversed. To accomplish this, a cross bar, the range of motion of which is limited by two adjustable stops, has been attached to the extreme right end of the guide bar shaft (only the top edge of the cross bar is visible in the photograph). The stops consist of eccentric discs which can be clamped in any position, so that the operator need not adjust the settings on the vernier and scale before each reversal of the knife, but merely shifts between the two fixed positions.

The 1.59 cm. shaft for the glass wheel is mounted on high quality bronze bearings spaced 15 cm. apart. The desirability of mounting the glass wheel on its own shaft with its own bearings rather than on the armature of the motor has been emphasized by Prof. I. W. Bailey of the Bussey Institution, and Prof. Long has informed the writer that his forthcoming model will be so equipped. The advantages of such an arrangement, already amply demonstrated in a machine built at this institution several years ago by Mr. W. F. Court, Technical Assistant, are fourfold. It enables the bearings to be more easily protected from the abrasive suspension, favors accuracy in alignment, lowers replacement costs should bearings become worn, and leaves the motor free for other purposes.

The question naturally arises as to what further improvements are feasible on the vertical wheel sharpener. Would it be practicable to make it automatic? The extreme complexity involved in such a modification, and therefore expense, as well as the expectations of performance, seem to point to a negative answer. Even in a well executed and perfectly functioning automatic device, it is necessary to rely on actual inspection of the knife edge under the microscope. This means the attention of an operator. Furthermore, for critical and/or difficult sectioning it is most desirable to have the sharpener constantly available for frequent reconditioning of the knife by a few polishing strokes. This is more readily accomplished on a simple apparatus. Two conclusions follow: first, there is no ultimate substitute for "a little mechanical skill", and second, the sharpening process must be controlled by the one who uses the knife. Today, as in the day of von Mohl (1857), the critical worker "wird selten Gelegenheiten finden, seine Messer durch einen andern gut schleifen zu lassen."

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A MODIFIED WRIGHT'S METHOD FOR STAINING BLOOD SMEARS

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Abstract.—**After the blood smear is treated for the proper length of time with Wright's stain, neutral distilled water is used for diluting the stain. After the slide has been treated with neutral distilled water until the smear becomes pinkish it is then treated with pure absolute methyl alcohol which destains the plasma. Neutral distilled water is again kept on the mount until the corpuscles are well stained. Then the slide is dehydrated with absolute ethyl alcohol, cleared with clove oil and completed in the usual way.**

Blood smears of different groups of vertebrates were uniformly brilliantly stained with the above technic.

Several lots of Wright's dry stain have been tested with the modified technic and no difficulties have been encountered in its application.

The well-known and commonly used Wright's stain for blood smears is a very satisfactory one. Sometimes there are difficulties, however, in securing uniformly successful results with the technic described by Wright (1902, 1910) and which have not heretofore, so far as I am aware, been overcome. Recently, Kingsley (1936) has referred to some of the unsuccessful results obtained with the Romanowsky (1890, 1891) types of blood technic, including Wright's, and has suggested a new stain for general hematological preparations.

The smears may be made in the usual way by placing a drop of blood on a clean slide and spreading it out by means of another slide, or a square cover glass. A much better method for making the smears has been used by the writer. A small sponge of fine texture is moistened with fresh blood and lightly drawn over the surface of a clean glass slide. With a little practice, it is possible to make a large number of good smears with a sponge which has been moistened only once with fresh blood.

Several lots of Wright's stain, including certified ones, have been tested by means of the modified technic, and all of them gave excellent results.

Blood smears of amphibians, reptiles, fish, birds, and mammals were made, and no difficulties were encountered with the technic.

Better results were obtained with the blood of certain amphibians with large corpuscles, such as necturus, when the smears, before dry-

ing, were exposed for about a minute over the fumes of a 2% osmic acid solution. Better results were also obtained with such smears, if the slides were placed immediately upon drying into a Coplin jar containing pure absolute methyl alcohol. After such treatment for a few minutes, the slides were rinsed in neutral distilled water and stained at once.

The various steps in the technic are as follows:

1. Place 4 or 5 drops of Wright's stain on slide with blood smear for one to two minutes.
2. Dilute the stain with an equal number of drops of neutral distilled water. Allow to remain for about three minutes.
3. Drain slide and flood it with neutral distilled water. Replace the water when the smear appears slightly pink. Drain off the water.
4. Place slide for a few minutes into a Coplin jar containing pure absolute methyl alcohol. Instead of placing the slide into a Coplin jar with the methyl alcohol, a few drops of the methyl alcohol may be placed on the smear.
5. Remove the absolute methyl alcohol with neutral distilled water. Replace with more of the distilled water, and allow to remain on the stained smear until a pinkish color appears. Remove portions of smear not desired. Examine slide.
6. Dehydrate with absolute ethyl alcohol.
7. Clear with clove oil.
8. Follow clearing with balsam.
9. Place cover slip on stained smear.

The use of neutral distilled water is very important. Ordinarily freshly distilled water was sufficiently neutral for use. The pure absolute methyl alcohol removes at once the plasma stain, hence, a much better preparation may be obtained. The use of the absolute ethyl alcohol and clove oil insures a much more brilliantly stained smear.

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THE ANILIN BLUE COLLAGEN STAIN

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The anilin blue method for staining collagen fibrils and reticulum was published in 1900.[1] Later (1905)[2] the phosphomolybdic acid, used originally by itself as a separate step, was combined with the anilin blue solution and the oxalic acid in it was omitted. In this form the stain has been in general use ever since.

The method has three defects in consequence of which it has been variously modified. Most of the acid fuchsins on the market tend to fade in the sections owing to the deleterious action on them of the phosphomolybdic acid. Moreover, the stain with the anilin blue is not always so intense and sharp as could be desired. The third defect was that the method did not give so good results with paraffin as with celloidin sections. The three modifications most in use are Heidenhain's azocarmine, the Lee-Brown, and the Masson trichrome methods. All are somewhat complicated.

Recently I have again modified the method by replacing the phosphomolybdic acid by phosphotungstic acid and prolonging the time of staining in the anilin blue solution. The acid fuchsin solution may be made weaker or stronger according to the intensity of the red stain desired. The result is a clean-cut, intense blue stain of the connective tissue fibrils, reticulum and basement membranes.

The method now advised for general purposes is as follows:

Fixation in Zenker's fluid.

1. Stain paraffin or celloidin sections in a .25% aqueous solution of acid fuchsin for 30 minutes.
2. Drain and pass sections directly to the following solution:

Water	100 cc.
Anilin blue W. S.	0.5 g.
Orange G (85–90% dye content)	2 g.
Phosphotungstic acid (Merck)	1 g.

Stain for 1 to 24 hours, or longer if the deepest color obtainable is desired. An hour in the paraffin oven gives as good a result as overnight in the cold.

[1]Mallory, F. B. A contribution to staining methods. J. Exp. Med., **5**, 15–20. 1900.

[2]Mallory, F. B. A contribution to the classification of tumors. J. Med. Research, **13**, (N. S. **8**) 113–36. 1905.

3. Transfer directly to 95% alcohol, two or three changes, until no more color is given off.

4. Absolute alcohol, xylol, neutral Canada balsam.

The method has been found to give excellent results with sections of formalin fixed pituitary glands. The acidophilic granules are stained bright red; the basophilic, blue.

TWO MICROTECHNICAL DEVICES

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1. A Simplified Mounting Cell

Microscopists frequently mount small, thick objects such as pieces of cleared bone, whole mounts of embryos, and many specimens of general zoölogical interest. For mounting such material two devices are available: excavated slides and glass rings. Since both of these are rather expensive, technicians sometimes make a crude substitute for the manufactured glass ring by cementing on a slide small pieces of broken glass in the shape of a compartment which can be filled with balsam and over which a cover glass can be placed. The objections to this method are the time and labor required and the tendency of the balsam to escape between the pieces of glass. The common white celluloid curtain ring makes a very satisfactory substitute for the manufactured glass ring. Such a curtain ring can be cemented to a slide, filled with balsam, the object to be mounted inserted, and a round cover slip placed over it. Rough or warped rings can be smoothed by rubbing them on the side of a flat file, or enough cement can be used to compensate for the unevenness. Thick balsam makes an adequate cement. The only part of the mounting procedure which requires practice is judging the amount of balsam needed to fill the ring exactly so that when the cover glass is put on there will be neither overflow of balsam nor a deficiency resulting in air bubbles. Curtain rings may be procured at any dry goods or department store at a price not to exceed five cents a dozen. The size which measures 1/8 inch in height, with inside and outside diameters of 5/8 and 7/8 of an inch, respectively, fits a 3/4-inch circular cover glass, and has proven very satisfactory.

2. A Container for Handling Nitrocellulose Sections in Coplin Jars

A desire for economy of both time and material when paraffin and nitrocellulose sections are being stained simultaneously prompted evolving a technic in which ordinary Coplin jars are used for dyeing nitrocellulose sections. The essential instrument is a perforated test tube which fits easily into the jars. A soft glass test tube 15 cm. long and 2 cm. in diameter is perforated with a hot 20-gauge platinum

[1]Contribution No. 230.

wire, altho hole size may be altered with variation in section size. If the holes are too large there is danger of the sections escaping. It is sufficient to have 5–12 holes near the bottom. One hole should be pierced at the very bottom of the tube to insure adequate drainage. Cracking of the tube may be avoided by heating and blowing the end enough to thin the glass prior to perforation. The sections to be stained are put into the perforated tube, and the latter is transferred from bath to bath. By wiping the tube and blowing the excess dye from it between jars, the contamination of the succeeding solution does not exceed that effected by a slide. As many as fifty sections have been stained at once with absolute uniformity of stain, even in short-time baths of high dye concentration. Staining homogeneity can be assured by stirring the solution in the tube with a suitable rod, or, more simply, by the application of gentle oral suction to the open end of the tube. Sections are most easily extracted from the tube by a stiff, flat paint brush $\frac{3}{8}$-inch in width.

A TECHNIC FOR CLEARING LARGE INSECTS

PAUL STAPP and RUSSELL W. CUMLEY, *Dept. of Zoology, University of Texas, Austin, Texas*

The following schedule has been found to clear large insects successfully. Highly pigmented animals and specimens with chitinous coverings are rendered transluscent by this method. Hence, finer structures of the external body wall may be studied to advantage.

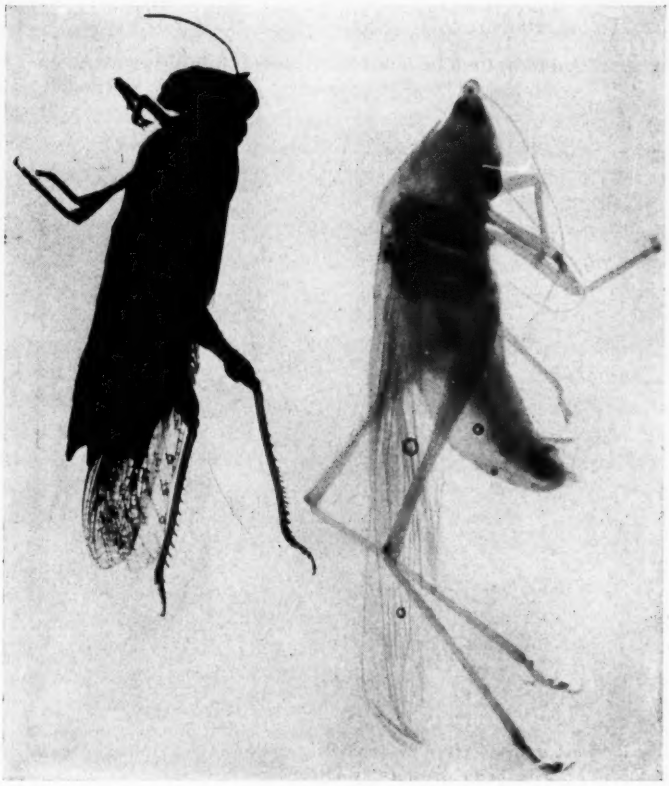

Fig. 1. Photograph showing two grasshoppers, the one on the left is untreated; the one on the right has been cleared by the technic described. Magnification approximately 2×.

Some dyes, e. g., picro carmine and borax carmine are not washed out by the various reagents, with the result that the organs which take the stain may be rendered more conspicuous. The two photographs demonstrate the effect produced by this technic.

The method is as follows:

1. Absolute alcohol, 5 to 15 days.
2. Alcohols, 95%, 85%, 70%, 50%, about 15 minutes each.
3. Alcohol, 35%, 30 minutes.
4. H_2O and H_2O_2 (50–50) plus trace of NH_4OH, 12 to 24 hours.
5. Alcohols, 35%, 50%, 85%, 95%, about 15 minutes each.
6. Absolute alcohol, 2 or 3 changes, at least 3 days.
7. Toluol, 10 days to 3 weeks.

The specimen may then be mounted in some suitable medium such as

Fig. 2. Photograph of a fruit fly, *Drosophila*, which has been cleared by the technic described. Magnification approximately 40×.

dammar. This is best accomplished by passing gradually from thin to thick dammar so that there is a thoro penetration of the medium. If the dammar is dissolved in xylol, the specimen should first be passed thru the latter before proceeding to the dammar. Benzole or xylol may be used in place of toluol, but either makes the material more brittle than does toluol.

If rings of gum rubber are cut and dropped in xylol for a short time, they become hard and afford a good support for cover glasses in the mounting of larger insects.

POSSIBLE USES OF DIOXAN IN BOTANICAL MICROTECHNIC

FRANK P. MCWHORTER and ELLIOT WEIER, *Oregon Experiment Station, Corvallis, Oregon*[1]

ABSTRACT.—**Dioxan has been well established as an advantageous dehydrating agent for plant tissues. It dehydrates equally well after fixatives containing formalin, acetic acid, chromic acid, chromates, mercuric chloride, osmic acid, and alcohol. Better infiltration of paraffin after dehydration may be obtained by passing the material thru (1) a cold bath composed of 30 cc. of dioxan, 5 cc. of xylol and 20 cc. of melted soft paraffin and, (2) a warm bath of 50 cc. of dioxan, 50 cc. of paraffin, and 10 cc. of xylol. Transfer from (2) to soft paraffin. A dioxan fixative consisting of dioxan 50 cc., formalin 6 cc., acetic acid 5 cc., water 50 cc. was devised for delicate subjects. The fixed material is transferred directly into dioxan and mounted in dioxan-diaphane or dioxan-balsam. Very delicate objects require dioxan dilution of the balsam and slow concentration of the mounting medium by evaporation.**

Entire plant parts or epidermal peelings are fixed in any desired fixative, washed if necessary, transferred to dioxan and mounted in diluted dioxan-balsam or diaphane. Dioxan may be used to mount hyalin objects whose refractive indexes approach those of balsam in media of higher index than balsam. It may be used in place of alcohol in finishing parafin sections, and since it exhibits different stain solubilities than alcohol it offers an important new tool in obtaining and maintaining stain balances.

Since the publications by Graupner and Weissberger (1931 and 1933) recommending the use of dioxan (diethylene-oxide)[2] as a substitute for alcohol and xylol in microtechnic processes a few American workers have observed the advantages of this chemical. Johansen (1935) discussed the desirability of dioxan and tertiary butyl alcohol as dehydrants for plant tissues but did not elaborate upon the details of his technic. Recently Baird (1936) discussed the application to animal tissues. From nearly two years' experience with dioxan in botanical microtechnic in this laboratory we feel that dioxan both simplifies and improves paraffin sectioning and that it offers new fields or possibilities in microtechnic. This paper presents schedules

[1]Published as Technical Paper No. 243 with the approval of the Director of the Oregon Experiment Station. Contribution from the Department of Botany.

[2]We obtained diethylene oxide from the Carbide and Chemical Corporation, under the name dioxan.

adaptable to plant tissue embedding, and points out some of the special applications of dioxan.

Schedules for the Paraffin Method

Fixation: We have used dioxan with a number of standard botanical fixatives comprising mixtures of formalin, acetic acid, chromic acid, and alcohol, and with a few cytological fixatives including Flemming's, Helly's, and certain mitochondrial technics. The choice of fixative does not limit the use of dioxan, but chromic acid mixtures do seem especially suitable for dioxan dehydration.

Washing: Except where tissue has been darkened (strong chromic or Flemming's) elaborate washing is unnecessary, rinsing off the excess fixative being sufficient. After washing or rinsing, transfer to undiluted dioxan.

Dioxan containers: When considerable embedding is to be done, the original recommendations of Graupner and Weissberger (1931) are advisable. The process consists of using the dioxan in a wide-mouthed glass-stoppered cylinder; the lower part of the cylinder is filled with anhydrous calcium chloride held in place with a fine-meshed wire screen. The principle involved is that the dioxan removes the water and fixative from the tissue and the calcium chloride removes both from the dioxan. One cylinder of dioxan and calcium-chloride will suffice for over one hundred embeddings. Tea-balls are convenient for holding the tissue in the liquid. When only a relatively small amount of embedding is to be done the somewhat "fussy" calcium chloride cylinders are unnecessary. In this case the dioxan is used as one would alcohol: simply place the fixed tissue (after a short washing) into a small vial, cover well with dioxan and change once or twice to insure complete dehydration. The cylinder method is advantageous and economical for handling large amounts of tissue, or for demonstrating the technic to students; the vial method for the individual worker who makes paraffin sections only occasionally. Dioxan containers should not be used or kept on any painted surface since *the chemical is an exceedingly active paint remover.*

Dehydration: After rinsing off the fixative pass the tissue directly into undiluted dioxan. In our tests grading dioxan as one would alcohol proved to be of no advantage. The time in dioxan will naturally vary with the size of the tissue. Three hours is a satisfactory time for small pieces not more than one millimeter thick, six hours for larger pieces. Two hours is the shortest time we have tried, but that was with very small pieces of tissue. Since tissue does not *materially* harden in dioxan one may leave the tissue in dioxan for several days without affecting the efficiency of the pro-

cess—a convenience to one who has to pick the time he can conveniently embed.

Embedding baths: While dioxan is only a weak solvent of paraffin, it is readily miscible with paraffin. One may transfer from dioxan directly to soft paraffin, but trial embeddings of thick leaf tissue have shown that it is advantageous to add a small amount of xylol at the beginning of the embedding series. Two special baths of paraffin mixtures have been standardized in this laboratory:

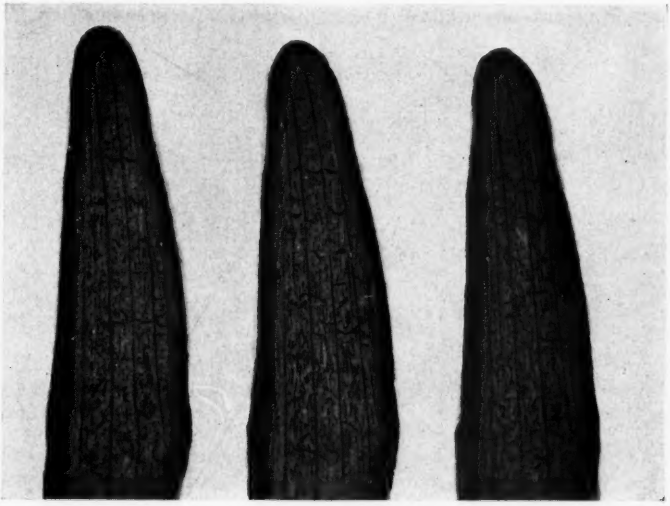

Fig. 1. Sections of narcissus leaf cut parallel to surface. These large sections cut with rotary microtone were possible because the leaf blade remained flat during the dioxan-paraffin embedding process. 72 mm. microtessar projection at 4 ×.

(1) To 50 cc. of dioxan and 5 cc. xylol add 20 cc. of melted, soft paraffin. This produces a flocculent-like mixture. Allow this mixture to stand until it assumes room temperature and forms a floating "cake". Then pour the liquid from under the paraffin into a suitable container. This becomes paraffin bath No. 1 and is used at room temperature. On cold days it may become turbid and should then be warmed before using.

(2) To 50 cc. of dioxan add 50 cc. of melted paraffin and 10 cc. of xylol. This bath is used at 50° C., or whatever temperature one chooses for soft paraffin.

Embedding procedure: Tissue is transferred quickly from dioxan into small vials in which paraffin mixture No. 1 is immediately poured.

The vials are placed in a vacuum chamber for 10 to 30 minutes. Mechanical removal of air greatly improves penetration into plant tissues where large air spaces are present even if the tissue was vacuumed during fixation. The total time in bath No. 1 need not be more than 30 minutes. The liquid (No. 1) is then poured off and bath No. 2 at 50° C. is quickly poured in, and the vials placed in the oven. The time in bath No. 2 need not be more than an hour.

From No. 2 transfer directly to pure soft paraffin, to hard paraffin if necessary, and embed as usual.

Some of the advantages of dioxan for the paraffin method are:

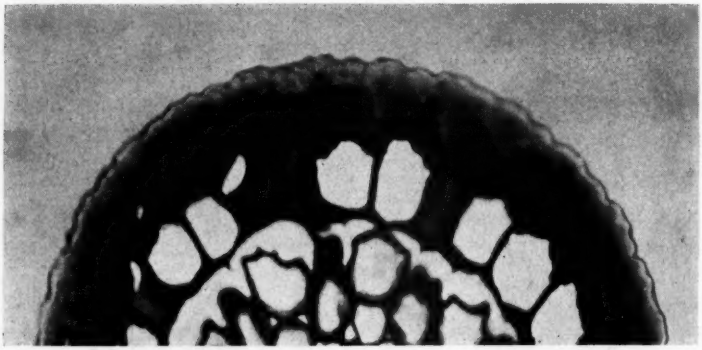

Fig. 2. Cross section (paraffin) of edge of leaf of Vallota, a thick-leaved member of the Amaryllis family. The solvent action of dioxan does not interfere with the preserving and recording of cuticular coverings. These thick-walled epidermal structures become brittle in alcohol-xylol embedding but are easily cut by the dioxan process. Stained in safranin. Photographed with B filter. Zeiss 8 mm. N. A. 0.65. (400 ×)

(1) Washing after fixation is largely eliminated. Dehydration and washing take place simultaneously. Graupner and Weissberger (1931) pointed out that even those fixatives containing picric and trichloracetic acid can be "washed out" with dioxan-calcium-chloride.

(2) The tedious alcohol-xylol gradation series is completely eliminated. There is only one step between fixation and infiltration. Infiltration is usually accomplished without any plasmolysis and with far less plasmolysis than the average carefully graded alcohol-xylol series permits.

(3) There is little hardening of tissues. Johansen (1935) attributes this to dehydration without desiccation. See Fig. 2.

(4) Tissues show much less tendency to curl than in alcohol and xylol. See Fig. 1.

(5) Speed and convenience: After fixation the tissue can be left

in dioxan for three hours or until one is ready to embed. Plant materials left over 12 hours become clear but do not harden.

(6) Material passed thru dioxan into paraffin can be softened in water for sectioning. A technician working under our direction cut thick canes of Ribes by this process. Johansen has noted this point and utilized it for cutting wood sections.

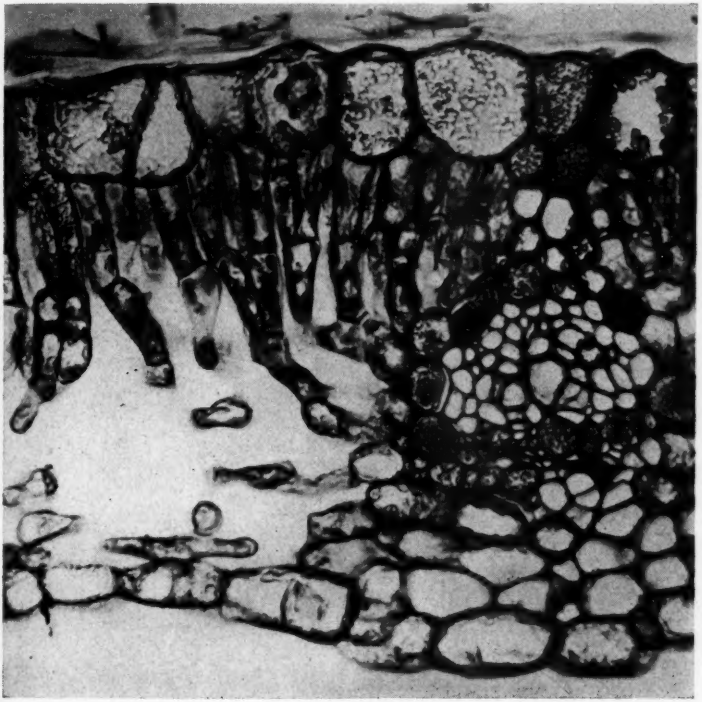

Fig. 3. Cross section (paraffin) rose leaf attacked by powdery mildew (Sphaerotheca). Two lobed-haustoria are visible in the epidermis. The epidermal cells and those of the pericycle contain gums and red pigments. This slide is faintly stained in safranin to facilitate rendering the subject in monochrome. Photograph shows how the extremely varied cell contents are retained during the dioxan process. B filter. Zeiss 4 mm. 0.95 N. A. (600×).

(7) It is an inexpensive method. One liter of dioxan is sufficient for at least one hundred embeddings.

Some of the disadvantages of dioxan for the paraffin method are:

(1) Very soft tissues remain too soft for satisfactory embedding. For example, partly ripened grapes remained too soft and failed to embed properly.

(2) Penetration by paraffin is not always satisfactory. This can usually be overcome by increasing the amount of xylol in the first paraffin bath. Tissues that are difficult to penetrate by the dioxan method are likewise difficult subjects for the standard alcohol-xylol method.

The above statements are based on the experience of making about 800 research slides representing about 160 separate embeddings, and on trials by students in technic courses.

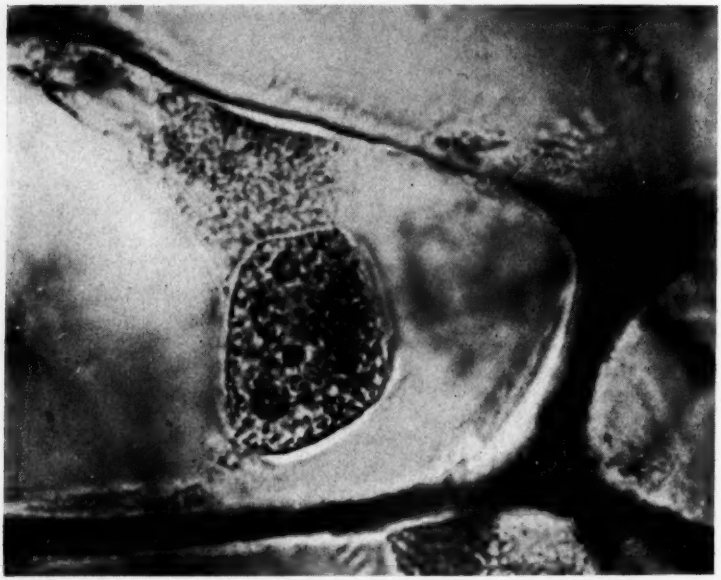

Fig. 4. Young "net" of the alga Hydrodyction mounted in Diaphane after fixing in dioxan fixative (page 111) and dehydrating in dioxan. Unstained. Photographed with Zeiss 3 mm. N. A. 1.00 lens. H filter, ⅓ light cone. (800 ×)

Special Uses and Methods

1. Mounting delicate microscopic subjects in balsam

Dioxan is an astoundingly efficient dehydrating agent for mounting delicate objects in balsam or balsam-like media. Johansen was the first to mention this use. We had independently developed this technic and concluded that such usage of dioxan is its most important and unique application. It was practically impossible, however, to fix some protozoa, the algae, Hydrodyction, and spores of fungi without slight plasmolysis with any fixative we tried. Dioxan treat-

ment did not increase the plasmolysis. This suggested the possibility of including dioxan in the fixative. The following formula was devised:

Dioxan	50 cc.
Formalin	6 cc.
Acetic acid	5 cc.
Water	50 cc.

This fixative has permitted balsam or diaphane mounts of Vorticella, Hydrodyction, Mucor, nematodes (several kinds) freehand

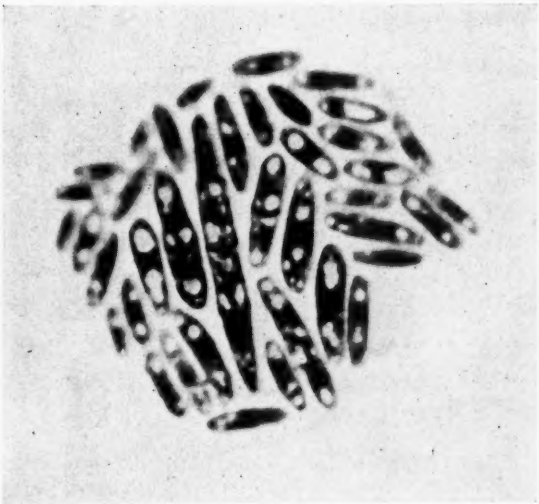

Fig. 5. Spores of Cylindrocarpon (a Fusarium-like fungus) from agar culture. Dioxan fixative, dioxan processed. Stained in Ehrlich's hematoxylin to demonstrate nuclear content. Late stages of mitosis are shown in the central spore. Zeiss 3 mm. 1.40 N. A. (1800 ×)

sections of mushrooms dropped directly into the fixative, mites, and many other subjects which are usually difficult to mount even in glycerin. In many of these preparations magnifications of 1,000 times fail to show a trace of plasmolysis. See Fig. 4 of Hydrodyction and Fig. 5 of fungus spores.

Fix ten minutes to one hour in dioxan fixative. Transfer to undiluted dioxan, or if advisable to a stain[3] dissolved in 50% dioxan,

[3]Staining methods for this particular purpose will require special investigation. Methyl blue in 50% dioxan is one of the most promising yet tried. Most stains are only slightly soluble in dioxan thus permitting dehydration with little stain removal. This factor makes it difficult to stain in dioxan without running the material back to water.

and then to pure dioxan. Place a few drops of very dilute Canada balsam dissolved in dioxan, or in diaphane diluted with dioxan, in a watch glass. For delicate subjects the mounting fluid must be water-like in dilution; use about ten parts of dioxan to one of balsam. Place specimens directly into this; allow the preparation to evaporate at room temperature until it assumes a syrup-like consistency. Transfer to a drop of dioxan-balsam or diaphane of about the same consistency and cover, adding thick balsam in dioxan to the edge of cover, as needed.

The presence of xylol in the balsam is objectionable for two reasons:

(a) If no xylol is present, traces of water which may have remained in the tissue, escape into the dioxan balsam. Dehydration continues after mounting!

(b) If the slide proves unsatisfactory and no xylol is present, the balsam is easily removable by washing in water!

Very small objects can be run thru on a slide or cover. We frequently make mounts of fungus cultures in the fixative instead of in water. Then if it seems desirable to preserve the mount, dioxan is drawn under the cover, then dioxan-balsam, and the mount is preserved.

2. MOUNTING DELICATE OBJECTS IN MEDIA OF HIGH REFRACTIVE INDEX

It is well known that mounting media frequently obscure details because the refractive index of the medium is too close to that of the object. This is especially true of glycerin preparations where the index ranges between 1.40 and 1.46. Sugar solutions such as "Karo" with an index around 1.48 are easy to use but have the same objection. Canada balsam, $1.50 \pm .02$ is slightly better but requires staining of objects for optimum vision even with critical illumination. Water mounts, with the index 1.33 well below that of the object, offer for critical illumination methods objective fields in which a hyalin object shines in a light ground—but such mounts are not permanent. By dioxan infiltration it is possible to mount objects in synthetic gums or resins such as "Hyrax" with a refractive index greater than 1.80. In such a medium objects which are hyalin in water appear as dark bodies on a light ground. At the present time we are having made special lots of "Hyrax" material for perfecting this method of mounting. It is probable that such a method will eliminate the necessity for staining where the form, shape, and size of the object are the chief considerations. These conditions obtain

in the average mycological preparation. The effect of the medium on resolving the outline of the object is shown photographically in Fig. 7.

3. MOUNTING ENTIRE PLANT PARTS AND EPIDERMAL PEELINGS

The process for mass tissues is essentially the same as that outlined in No. 1 but for some reason tissues are far less prone to plasmolyze than filamentous fungi and algae. They may, therefore, be handled with fewer precautions and with a larger variety of fixatives. The following procedure is efficient and rapid for soft plant tissues such as portions of leaves or slices of herbaceous stems and roots.

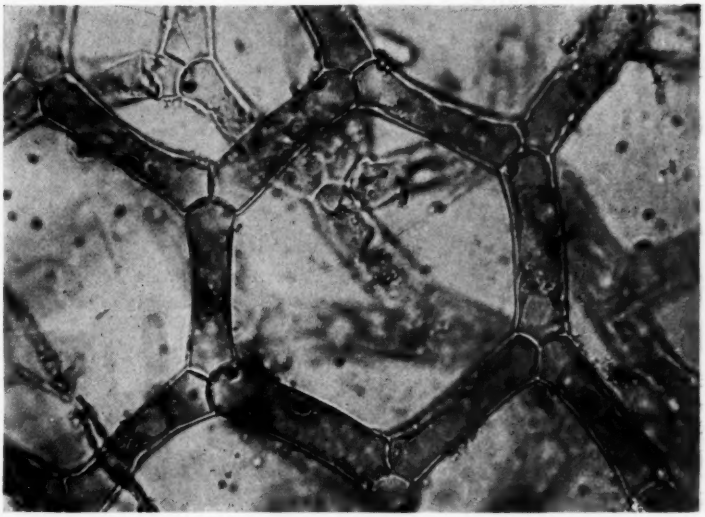

Fig. 6. The whole mount method applied to x-body differentiation. Subject, *Hippeastrum equestre*, virus diseased (type material plants supplied by Dr. Kunkel) leaves sliced parallel to epidermis, (i. e. "chunks," not machine sections) fixed in aceto-formalin, faintly stained in Ehrlich's hematoxylin (after mordanting as for iron hematoxylin) counterstained in gentian violet dissolved in 50% dioxan, transferred to pure dioxan, cleared (in dioxan) for 18 hours, then mounted in Diaphane diluted with dioxan. Photographed: Zeiss 3 mm., 1.40 N. A. on process pan. The picture represents an optical section of the nucleus and its plasmosome. Chromatin granules of the resting nucleus are clearly shown. The tail-like material subtended by the nucleus is a partly organized x-body. (1200×)

Fix in any desirable fixative. Five per cent acetic acid, 6% formalin in 50% alcohol, *mixed when used* to avoid formation of esters, is usable for almost any tissues.

Transfer directly to pure dioxan or to a stain, and then to dioxan. The minimum time in dioxan is 30 minutes, but if the tissue is left for several hours it will become very clear and permit better vision of the inner cells.

Mount in dilute dioxan-balsam or diaphane. It is not necessary to use very thin balsam and evaporate it down in this case.

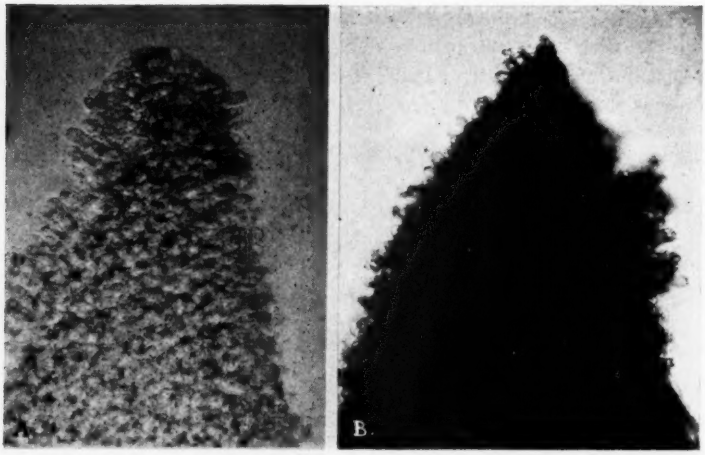

Fig. 7. Photographs of small bits of mushroom (Leptonia) gill mashed flat, fixed in dioxan-fixative and dioxan dehydrated. (A) mounted in Canada balsam (Diaphane) (B) mounted in Hyrax. These are photographed with a Zeiss 8 mm. 0.65 N. A. lens using exactly the same light cone ($\frac{2}{3}$) in each case and commercial Panchromatic film. The difference in appearance and in rendering of basidia at the edges is due entirely to the refractive index of the medium. Neither are stained and the tissue is very hyalin. This high refractive index medium (above 1.80) were designed for diatom shells and similar non-plasmolizable material, but by dioxan processing they may be used to show the form of delicate hyalin objects. Prints on "contrast" vitava paper carefully balanced to show relative contrast of negatives. (400 ×)

This method is especially useful for leaves. Cytological details may be readily followed and gross anatomy is visually charted. If stained in light green, they appear as living entities, but are more visible within than are living tissues because of the clearing. Permanent mounts of epidermal peelings and portions of leaves have proven efficient for studying x-body inclusions within tissues from virus diseased plants. (Fig. 6).

4. MITOCHONDRIAL AND PLASTID TECHNIC

Dioxan is efficient in connection with mitochondrial technics. Altho a powerful solvent of fats and lipoids, it does not appear to remove them from cells where they have been subjected to saponification with chromates. The usual procedures of fixation are followed, the material is washed and then dehydrated in dioxan. Cytological details within growing points and leaves are well preserved.

Epidermal peels fixed in mitochondrial fixatives, washed, stained, dehydrated in dioxan and mounted in balsam-dioxan give excellent preparations of plastids without recourse to tedious alcohol dehydration or to paraffin sectioning. Other than special fixation the technic is essentially that presented in No. 3, above.

5. RUNNING SECTIONS DOWN FROM XYLOL AND STAINING

Dioxan can be used to replace most of the alcohol series in running paraffin sections down from xylol. The important difference in stain solubility existing between alcohol and dioxan will permit the development of new stain technics and simplification of existing technics where the extreme solubility of some stains in alcohol causes difficulties in finishing slides. These stain solubility differences offer an important field for investigation.[4]

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————. 1933. Die Verwendung von Lösungen in Dioxan als Fixierungsmittel für gefrierschnitte. Zool. Anzeiger, **102**, 39–44. (English translations of both the original German articles are obtainable from the Carbide and Carbon Chemical Corporation. The second article should be of special use to animal histologists interested in ultra-rapid methods of processing frozen sections.)

JOHANSEN, D. A. 1935. Dehydration and infiltration. Science, **82**, 253–4.

[4]In the synthetic organic chemical catalog of Carbide and Carbon Chemicals Corporation it is stated "The oil soluble dyes are soluble in dioxan while the water-soluble and spirit-soluble varieties are practically insoluble."

A NEW STAINING TECHNIC FOR PERITHECIA OF THE ERYSIPHACEAE

GORDON D. ALCORN and CLAIRE L. WORLEY, *Univ. of Idaho, Moscow, Idaho*[1]

Any process involved in the staining of perithecia should be simple and yet satisfactory from the standpoint of detail.

It was our idea that a good bacteriological stain designed for the penetration of hardened cell walls of certain bacteria could likewise be used to penetrate peridial walls. While the bacteriologist uses a short-period heating process for penetration of stain, we used a long-period process (48 or more hours). Our theories regarding stain penetration seem to be justified as the results desired were all present. In the following schedule perithecial wall, asci, ascospores, nuclei, and even appendages were well stained. Care in handling will eliminate danger of breaking off appendages.

Dry perithecia carefully scraped from the leaf of the host should be placed directly in Ziehl's carbol fuchsin for a period of 48 hours or more, in an oven kept at 50° C. Some of the perithecia carefully removed from the staining vial should be placed on a clean slide and covered, and the perithecia opened by tapping, smartly, on the cover slip. Slides bearing opened perithecia should then be heated for 15 minutes in an oven kept at 95° C., with stain constantly being added to replace that being steamed off. A drop or two every three or four minutes placed at the edge of the cover slip will suffice. Excess stain around the cover slip should be carefully removed before the destaining process begins. For destaining acid alcohol (2% solution of concentrated HCl in 95% alcohol) must be drawn rapidly under the cover glass by means of a blotter or filter paper. We have found that six to ten drops of acid alcohol will suffice for destaining, after which the stained organisms are washed by allowing 95% alcohol to be drawn under the cover slip. The process is complete after the perithecia are washed in a series of 25%, 50%, and 75% xylene in 95% alcohol, followed by pure xylene. Since the perithecia adhere to the slide, the cover slip may be removed while the balsam or hyrax is being added.

DETAILS OF TECHNIC

1. Dry perithecia should be stained in Ziehl's carbol fuchsin (fuchsin 1 g., carbolic acid crystal, 5 g., 95% alcohol 10 cc., H_2O 100 cc.) for a minimum of 48 hours in an oven at 50° C.

[1]Mr. Glenn L. Hays aided in preparation of stain.

2. Place perithecia on a slide and open with cover slip.

3. Transfer to oven for 15 minutes at 95° C.; evaporating stain to be replaced by more stain.

4. Remove with acid alcohol (2% solution of concentrated HCl in 95% alcohol) all excess stain left on the slide.

5. Destain with acid alcohol; it is well to watch this process under the microscope.

6. Wash thoroly with 95% alcohol.

7. Wash with 25%, 50%, and 75% xylene in 95% alcohol, followed by pure xylene.

8. Mount in balsam or hyrax.

NOTES ON TECHNIC

A Dioxan Technic.—In recent years the classical technic in cytology has undergone some important changes which have tended not only to hasten processes but to improve results. These advances have been made possible thru the use of two substances, i. e., butyl alcohol and dioxan. The latter substance has some valuable properties which give promise of making it one of the most important agents in microscopical technic. Recently I have extended the use of dioxan and have thereby been able to simplify and shorten the process of making sections by the paraffin method. In this new technic the tissue never leaves dioxan solutions, unless for staining, from fixation until embedding and after sectioning is mounted in a dioxan medium. Changes are reduced to a minimum and treatment with harsh media is avoided, thus lessening hardening and shrinkage of delicate materials. Briefly the steps are as follows:

1. Fix in a mixture of one part of dioxan with 2 parts of a picroformol-acetic solution (e. g., Bouin, or one of the writer's modifications thereof.)
2. Wash in dioxan.
3. Dehydrate and clear in fresh dioxan.
4. Infiltrate with warm mixtures of paraffin and dioxan, with increasing proportions of paraffin.
5. Infiltrate in paraffin.
6. Cut, spread and dry sections.
7. Decerate in xylene or dioxan.
8. Stain.
9. Wash.
10. Dehydrate with dioxan.
11. Mount in sandarac dissolved in dioxan, the concentration varying with the type of tissue, and to be determined by experience.

Dioxan dissolves picric acid readily, but does not dissolve many of the anilin dyes tried. It is a partial solvent for water, alcohol, xylol, paraffin, sandarac and dammar. The milky solution of dammar can be cleared by the addition of a quantity of xylol. By the addition of camsal (a fluid produced from a mixture of camphor and salol) to the sandarac solution in dioxan, a mounting medium similar to euparal is produced. Material can be left in dioxan for several weeks without injury. Infiltration in paraffin is slower after dioxan than after xylol, but tissues do not suffer from the heated paraffin.

Dioxan is more expensive than alcohol, but by making use of a series of baths very little need be lost and in this way it costs no more to use than ordinary reagents. By using fixing fluids with dioxan, acetic acid and chloroform, or with dioxan, acetic acid and formalin, a quicker result can be obtained than with picric acid. Fixed materials stored in 70% alcohol can, of course, be carried up into dioxan, sectioned and mounted according to the suggested method.—C. E. McClung, Univ. of Pennsylvania, Philadelphia.

The Reflecting Prism in Microscopy.—H. C. Waterman, in his notes in Stain Technology (January, 1934), deplores the prohibitive price of the reflecting prism recommended by Belling in place of the microscope mirror. Thrifty readers may, perhaps, benefit by my own experience. Recently, I noticed a heterogeneous collection of prisms in an optician's window in England which, I was informed, had been salvaged from derelict instruments. I had no difficulty in securing a perfect specimen of ample dimensions for the equivalent of two dollars. It seems probable that many opticians carry similar collections.—D. B. O. Savile, Macdonald College, P. Q., Canada.

A Simplification of the Paraffin Embedding Process.—The use of the electric refrigerator and rubber ice-cube trays in embedding with paraffin has been a means of saving time and obtaining more translucent blocks. The rubber type of tray is more practical than the metal type since it is pliable enough to allow the removal of the blocks after hardening. Altho they form only one size of block, this is large enough to contain good sized specimens, and yet does not form a permanent waste of paraffin around the smaller ones, since paraffin shreds may be remelted if the clearing agent has been removed before embedding.

The use of the electric refrigerator allows the regulating of the temperature needed to cool the blocks quickly and evenly. Small paper labels floated on the hot paraffin in the trays form adequate means of identification for each block.—Harris P. Mosher and Miss E. C. Adams, Mosher Laboratory, Massachusetts Eye and Ear Infirmary, Boston, Mass.

A Word of Caution Concerning Dioxan.—Since the publication of the article "Comparative Study of Dehydration", (Stain Technology, 11, 13–22, 1936), the attention of the author has been directed to the possible toxicity of dioxan. The Industrial Chemist, (No. 122, 11, 1935) states that the effects of inhaling dioxan are cumulative and result in pathological damage to the liver and kid-

neys. Prolonged exposure in concentrations 1 in 1000 produce severe toxic effects.

Caution in the use of this reagent is, therefore, necessary. It is not likely, however, that such industrial conditions will be duplicated in the technical laboratory where only small quantities of the reagent are used and mostly in closed bottles.—T. T. Baird, Columbus, Ohio.

STAINS RECENTLY CERTIFIED

In the table below is given a list of the batches of stain approved since the last one listed in the April number of this journal.

STAINS CERTIFIED MARCH 1, 1936 TO MAY 31, 1936*

Name of dye	Certification No. of batch	Dye Content	Objects of tests made by Commission†	Date approved
Basic fuchsin	DF 4	91%	For use in bacteriological media	Mar. 6, 1936
Congo red	LQ 5	75%	As histological counterstain	Mar. 12, 1936
Basic fuchsin	NF 28	84%	As bacterial stain and for use in bacteriological media	Mar. 13, 1936
Methyl violet 2B	NMv 5	85%	As bacteriological, histological and cytological stain	Apr. 1, 1936
Basic fuchsin	NF 3	91%	As bacterial stain and for use in bacteriological media	Apr. 1, 1936
Giemsa stain	GGe 3	——	As blood stain	Apr. 17, 1936
Janus green B	CJ 3	56%	As vital blood stain	Apr. 17, 1936
Giemsa stain	NGe 2	——	As blood stain	Apr. 21, 1936
Crystal violet	LC 10	92%	As bacteriological, histological and cytological stain	May 5, 1936
Eosin B	NEb 7	90%	As histological counterstain	May 6, 1936
Methylene blue	NA 11	86%	As histological and bacteriological stain; and as constituent of blood stains	May 22, 1936

*The name of the company submitting any one of these dyes will be furnished on request.

†It is not to be inferred that these are the only uses for which each of these samples may be employed. The Commission ordinarily tests each dye for such of its common uses as seem to give the most severe check as to its staining value. Certification does not in any instance, however, imply approval for medicinal use.

LABORATORY HINTS
FROM THE LITERATURE

A DEPARTMENT DEVOTED TO ABSTRACTS OF BOOKS AND PAPERS FROM OTHER JOURNALS DEALING WITH STAINS AND MICROSCOPIC TECHNIC IN GENERAL

The abstracts given here are intended primarily for laboratory use; consequently the technic in each instance is given in as much detail as possible.

J. A. de Tomasi — *Abstract Editor*

MICROSCOPE AND OTHER APPARATUS

HERTIG, M. **Hollow-ground slides for whole mounts made with the dental engine.** *Science,* **83,** 110. 1936.

The dental engine (motor, flexible shaft and grinding tools) proves to be a useful implement for making slides with all manner of depressions to fit any specimen. This permits resting the cover glass upon the slide, allows saving of the mounting medium and facilitates orientation of the specimen. Of the tools, the abrasive wheels mounted on a mandrel are perhaps the most generally useful. Grinding may be done rapidly in a drop of water. A depression for a mature flea larva will take 10–15 sec., for a mosquito larva 30 sec., for a bedbug 1 min.—*J. A. de Tomasi.*

SCHUMACHER, J. **Eine einfache Methode zur Angleichung der Bild- und Zeichenflächenhelligkeit bei Zeichenapparaten und -okularen.** *Zts. wiss Mikr.,* **51,** 392–3. 1935.

While sketching at the microscope, the usual way of equalizing the intensity of illumination of the microscopic field and the sheet of drawing paper consists in the interpolation of gray filters. This is often unsatisfactory, owing to the crudeness and other shortcomings of the filters. It is suggested that one extra lamp be supplied for the drawing board, with a variable resistance placed in the line at the left of the microscope. During the process of drawing, with the camera lucida in position, the left hand can operate the resistance knob so that the lighting can be equalized at will. Increasing or decreasing the illumination of the sketch also allows immediate comparison of the object and the drawing.—*J. A. de Tomasi.*

PHOTOMICROGRAPHY

BERTHELSEN, H. **Eine objektive Methode zur Bestimmung der genauen Expositionszeit bei der Mikrophotographie.** *Zts. wiss. Mikr.,* **51,** 383–7. 1935.

A method is described for the determination of the correct exposure time in photomicrography. It is based on the use of a photoelectric exposuremeter, the "Metraphot" built by Metrawatt Nürnberg A. G. The apparatus cannot give a direct reading at the microscope, but the exposure may be calculated by correlation. It is claimed the method affords a saving of time and plates.—*J. A. de Tomasi.*

JORDAN, H. **A new apparatus for the daylight projection of microscopic and lantern slides.** *Science,* **83,** 167–8. 1936.

This simple screen or reflecting box permits projection of lantern slides, photographs, and plates in plain daylight. It consists of a box with sides sloping outwards, its farther face covered with glazed or aluminum-coated paper. The rest of the interior is painted black. It has proved of value in demonstrating to small groups of students.—*J. A. de Tomasi.*

MICROTECHNIC IN GENERAL

CASTELLENGO, L. **Bobine pour rubans de coupes en série.** *Bull. d'Histol. Appl.*, **12**, 323–8. 1935.

This appliance consists essentially of a spool, controlled by a drum and axle, which is held with screws and brackets parallel to the microtome blade. The ribbon is wound by hand around the spool as soon as made. There are several advantages claimed, chiefly the production of continuous ribbons which should eliminate variations in thickness and loss of any sections. A series of 248 sections, 7 μ thick, 8 mm. wide, was cut in 48° C. paraffin. In spite of the 2 m. length of the ribbon and of 23° C. room temperature, no inconvenience was experienced.—*J. A. de Tomasi.*

COX, A. J., JR. **A simple basket carrier for use in tissue dehydration.** *J. Lab. & Clin. Med.*, **20**, 1298–99. 1935.

Cut out a piece of copper window screen 3½ x 2½ in., and a smaller piece about 1½ in. square. Two wires are removed from each end of the large piece. Roll this into a cylindrical shape, interlace and tie the protruding ends. Remove two wires from one end of the cylinder thus formed and attach the smaller piece of screen to make the bottom. The resulting basket measures 1 x 2¼ in., is quite cheap, and can be used for fixation as well as embedding.—*J. A. de Tomasi.*

ETKIN, W. **A simplified procedure for the volumetric measurement of serially sectioned structures.** *Science*, **83**, 214. 1936.

The volume of small or diffuse glands can be successfully computed by drawing (with the aid of a projector or a camera lucida) the outline of sections in sequence on a continuous sheet of wrapping paper. A draftsman's planimeter is set upon an ample sheet of clear flat material such as celluloid. A pinhole at the center marks the starting point The sheet with outlines is slipped under the celluloid and a point on each perimeter is brought to coincide with the starting point. Without disturbing the planimeter the pointer is traced around and the final reading gives the total sum of the areas. From this and the thickness of the section, the volume is calculated. The volume of a gland spread over more than 100 sections can be estimated in less than 2 hr.—*J. A. de Tomasi.*

GOOSMANN, C. **Dark-field illumination in the diagnosis of tuberculosis and malaria.** *J. Lab. & Clin. Med.*, **21**, 421–4. 1936.

Dark-field illumination is not only useful for the study of live organisms, but can also find application in the examination of stained slides. Thus tubercle bacilli appear much brighter and bigger, while the blue stained non-acid-fast bodies are almost invisible. The same is true of the malaria plasmodium in Giemsa stained blood smears. It should be easy to switch from dark-field to transmitted light illumination: all that is needed for this is a "center stop" to be inserted below the Abbe condensor. The field is searched with a dry condensor; a drop of water between condensor and slide will then give a better dark-field. As the size of the center stop depends on the aperture of the objective and on the ocular, it is well to keep several sizes on hand. The "Mikropolychromar" of recent introduction is based on the same principle: the dark center disc gives a blue background, while the lighter rim colors the objects bright red.—*J. A. de Tomasi.*

GRAFF, J. H., ULM, R. W. K., and HOWELLS, T. A. **The factors involved in the accuracy of fiber analysis.** *Paper Trade J.*, **101**, No. 2. 1935.

A standard method is given for preparation of slides for fiber anlysis of paper and making the fiber count. Results are accurate within less than 2% when two fields are counted, and should be reported in exact figures, not to the nearest 5%.

Cover 0.5 g. of the paper with 1% NaOH in a 30 cc. beaker and bring to boil on a hot plate. Decant and wash twice with water. Cover with 0.2 *N* HCl, let stand several minutes, decant and wash several times with water. Drain, roll into pellets and disintegrate by shaking in a flask with 250 cc. water. Measure 125 cc. of the suspension into a 250 cc. graduated cylinder, dilute with water to 250 cc. and mix. Pour off 125 cc., dilute again to 250 cc. and mix. This makes a 0.05% suspension.

Slides kept in 0.5% HCl in 50% alcohol are dried and polished. With a wax pencil draw a line across the slide 1 in. from each end to keep the suspension within end areas. Place on a level hot plate regulated to 50° to 60° C. Pipet 0.5 cc. of suspension onto each end of the slide. Evaporate partly, and tap to distribute uniformly. Then dry completely. Remove and add 2 to 3 drops of stain (particular stain not given); cover; let stand 1 min.; and drain on blotting paper.

Count intercepts of fibers with cross hair on 5 traverses across the slide 5 mm. apart, beginning 3 mm. from either end. Fine fragments are neglected, larger ones are counted as ½ or ⅓. Where 2 fibers are being counted, count both while going in one direction, but only one in the reverse direction. Determine second fiber by difference.—*E. Venable.*

KERNOHAN, J. W. **Methods of temporarily preserving fresh frozen sections stained with polychrome methylene blue.** *Amer. J. Clin. Path.*, **6**, 195. 1936.

The tissue coloration produced by polychrome methylene blue usually lasts only 1–2 hrs. in fresh frozen sections. By painting a clear, quick-drying lacquer (*Duco* from E. I. duPont de Nemours Co.) about the edge of the coverslip before the fluid dries, the color can be preserved several days or even weeks. This permits comparison between preserved and fresh tissues.—*V. Warbritton.*

PATRICK, R. **"Karo" as a mounting medium.** *Science*, **83**, 85–6. 1936.

"Karo", a commercial corn syrup containing maltose, dextrose and dextrin, can be used to advantage in mounting algae, pollen grains, and whole insects. Delicate forms require a conc. soln. Advantages: preparations are more permanent than in glycerin jelly; clearing is unnecessary; material can be mounted in it directly from water or lower alcohols; no ringing is necessary; none of the sugars will crystallize out.—*J. A. de Tomasi.*

RUIJTER, J. H. C. **Eine Methode zum Umranden von Präparaten.** *Zts. wiss. Mikr.*, **51**, 374–5. 1935.

Ringing of cover glasses with Noyer's lake is annoying when cedar oil is to be cleaned from the slide after observation with an immersion objective. Solvents of the oil are harmful to the lake. The following makes use of a photochemical reaction and works well: 20% gelatin in sat. thymol-water, 100 cc.; 5% $K_2Cr_2O_7$, 10 cc. Keep in the dark. When needed, melt it at 37° C. and distribute with a small brush. When dried in daylight the gelatin is coagulated irreversably by action of the light, and cannot be removed by any common solvents.—*J. A. de Tomasi.*

SCHMELZER, W. **Hecheln und Raspeln, angewandt auf tierische Gewebe.** *Zts. wiss. Mikr.*, **51**, 516–8. 1935.

Two ways are indicated for teasing and comminuting tissue in order to separate fiber and cell elements. For teasing, it is suggested that a pair of hackle combs be made by securing, for each, 6 medium size sewing needles at the end of a short piece of metal tubing; this end is pressed flat. One of these combs is used to hold the material fast, the other is brought to interlace with the former's prongs and then drawn away from it. The second operation is performed by circular motion, having the material suspended in 1–2 drops of glycerin between 2 pieces of sandpaper.—*J. A. de Tomasi.*

DYES AND THEIR BIOLOGICAL USES

BROOKS, M. M. **Mechanism of methylene blue in CO-poisoning.** *Proc. Soc. Exp. Biol. & Med.*, **34**, 48–9. 1936.

Using spectrophotometric methods on blood from rabbits poisoned with CO and subsequently treated with methylene blue, 0.03% in 0.9% NaCl, 1 cc. per Kg., results indicated that the dye changed CO-hemoglobin into oxyhemoglobin in the blood stream, and not into methemoglobin.—*M. S. Marshall.*

CLARA, M. **Über die Diazokuppelungsreaktion zum Nachweis der ortho- und para-Phenole in der histologischen Technik.** *Zts. wiss. Mikr.*, **51**, 316–37. 1935.

The colorless diazonium salts display the property of combining with phenols, phenolethers, and amino derivatives of aromatic compounds thereby forming colored bodies, the azo dyes. While this reaction can be carried out during the process of staining, the procedure of preparing a suitable diazonium salt is rather cumbersome for the average biologist. The use of already diazotized compounds simplifies the procedure. Experiments with "Nitrosaminrot" and "Nitrazol CF" (Hollborn, Leipzig) show that neither has practical value as they quickly deteriorate. The I. G. Farbenindustrie A. G. has prepared for the author a stabilized product "Echtrotsalz B" which is quite satisfactory. A small amount dissolved in ice-cold water is alkalinized with Li_2CO_3. Sections treated for 30 sec. and rinsed in water stain a light yellow, except the histologic structures containing phenol derivatives, which display a brilliant brown-red tone.—*J. A. de Tomasi.*

FRIEDMAN, M. M. and AUERBACH, O. **An improved Congo red test for amyloidosis.** *J. Lab. & Clin. Med.*, **21**, 93–4. 1935.

By intravenous injection of 10 cc. 1% aq. Congo red, a pathological condition like amyloidosis can be detected, thereby supporting clinical findings. The test is based on the theory that the dye can be recovered quantitatively from the blood serum of normal people; a loss of dye indicates absorption due to amyloidosis. Comparative figures are obtained by the use of the colorimeter, making certain that the serum has not been contaminated by hemolysis. The improvement introduced consists in the use of ethyl alcohol which precipitates the proteins and dissolves the dye, yielding a clear solution suitable for examination.—*J. A. de Tomasi.*

IDE-ROZAS, A. **Zwei neue Färbungsmethoden mit Hämatoxylin.** *Zts. wiss. Mikr.*, **52**, 1–7. 1935.

A report is given on the practical development of a new hematoxylin stain called "Hämatein nach Ide-Rozas", handled by Dr. G. Grübler & Co., Leipzig. It is made up fundamentally of a hematein lake in an $AlCl_3$ solution. It can be used after all common fixatives, is slow enough so that its staining can be controlled, and does not spoil in solution even in 2 years. A new formula for an iron hematoxylin is as follows: Weigert's hematoxylin, 15 cc.; double dist. glycerin, 5 cc.; iron alum-$AlCl_3$ solution (5% iron alum and 6% $AlCl_3$ in dist. water), 5 cc. (The Weigert hematoxylin is prepared by dissolving 1 g. hematoxylin in 10 cc. abs. alcohol and diluting to 100 cc. with dist. water.) Stain 12–24 hr. and differentiate in 2–2.5% iron alum. The staining appears to be much more uniform thruout a section in spite of accidental variations in its thickness.—*J. A. de Tomasi.*

KAWAMURA, R. and YASAKI, T. **Ueber eine neue Fettfärbungsmethode.** *Zentbl. allg. Path.*, **64**, 177–81. 1936. **Noch ein Wort zu Unserer Fettfärbungsmethode und über Modification in Herstellung der Stammlösung.** *Zentbl. allg. Path.*, **64**, 181–3. 1936.

An excellent review is given in these two papers of the present status of fat staining. The following improved Sudan III method is recommended. Grind 4 g. Sudan III (Grübler, Kahlbaum, or Ciba) in a mortar and add to the ground dye 450 cc. of 82% alcohol. Stopper the container loosely and heat slowly until the solution just begins to boil. Filter quickly while hot, set in a refrigerator for 12–24 hr., and filter a second time. This is the *stock solution.* To prepare the *staining solution* take 50 cc. of the stock solution add successive 2 cc. quantities, shaking about 20 times after each addition, until the final volume is 100 cc. Let stand 12–24 hr. at room temp. and filter. The pH should be 5.4 to 5.6.—*H. A. Davenport.*

LIN, F. C. **Photodynamic action of methylene blue on diphtheria toxin.** *Proc. Soc. Exp. Biol. & Med.*, **33**, 337–8. 1935.

Methylene blue, in 0.9% NaCl, in dilution from 1:100 to 1:100,000 with diphtheria toxin, when exposed either to sunlight or to artificial light, brought about marked reduction in toxicity of the toxin.—*M. S. Marshall.*

LISON, L. **Sur le mécanisme et la signification de la coloration des lipides par le bleu de Nil.** *Bull. d'Histol. Appl.*, **12**, 279–89. 1935.

It is understood that Nile blue sulfate can hydrolize, but only in solutions of high dilution; the greater the concentration the less the hydrolysis, in accordance with the mass law. From a dilute solution any fat solvent will extract an orange-red fraction called here "Nile red". It is found that the free base of the dye (prepared by alkalinizing, extracting with toluene, drying and redissolving in alcohol) does not stain fatty materials. The Nile red instead is the fraction responsible for the pink stain imparted to many substances. Commercial Nile blue always contains Nile red beside the true blue. Both of them stain fatty substances without displaying a definite specificity.—*J. A. de Tomasi.*

MICHAELIS, L. and SMYTHE, C. V. **Influence of certain dye-stuffs on fermentation and respiration of yeast extract.** *Proc. Soc. Exp. Biol. & Med.*, **33**, 127–9. 1935.

To an actively reducing yeast extract were added various dyes, including 1-naphthol 2-sulfonate indophenol, thionin, pyocyanin, indigo disulfonate, and pheno-safranin. The result was frequently an initial acceleration of fermentation followed by inhibition, but this inhibition did not correlate with oxygen consumption. It was irreversible and was due to destruction of enzymes.—*M. S. Marshall.*

STOWE, W. P. and DELPRAT, G. D. **The rose bengal test of liver function: photelometric method.** *J. Lab. & Clin. Med.*, **20**, 1297–8. 1935.

Rose bengal in 1% normal saline solution is injected intravenously for the comparative test of blood in a colorimeter. Correct reading is secured by means of a photoelectric cell, which eliminates the personal equation error. Methods of operation and calculation of results are given, but as they have no direct bearing on microtechnic, are not detailed here.—*J. A. de Tomasi.*

ANIMAL MICROTECHNIC

BECKER, W. S., PRAVER, L. L. and THATCHER, H. **An improved (paraffin section) method for the Dopa reaction.** *Arch. Dermat. and Syphilol.*, **31**, 190–5. 1935.

A paraffin method is given for the Dopa reaction which is superior to the frozen section method. Immerse fresh tissue 3–5 mm. thick in a solution of di-oxyphenylalanine "prepared according to the simplified technic of Laidlaw and Blackberg" (*Amer. J. Path.*, **8**, 491, 1932). Change the solution after ½ hr. The reaction of the solutions varies from pH 7.35 to 7.8. Incubate the solution containing the tissue at 37° C. for 12–15 hr. During this time the tissue and the solution turn black. Fix the block in Bouin's solution "according to the method of Masson" for 48–72 hr. Wash and dehydrate in alcohol; pass thru toluene; embed in paraffin containing 5% beeswax. Cut sections 5–6 μ and follow the usual technic down to water. Wash in running water for 1 hr. The trinitrophenol washes out and the collagen appears gray. For a simple counterstain brazilin may be used. For cytological study use the Masson trichrome stain (iron hematoxylin, acid fuchsin-ponceau de xylidine and anilin blue) as a counterstain. Identification of the Dopa-positive cells is enhanced by a light staining of the protoplasm with the acid fuchsin-ponceau de xylidine mixture.—*M. Lois Calhoun.*

BLAIR, D. M. and DAVIES, F. **Observations on the conducting system of the heart.** *J. Anat.*, **69**, 303. 1935.

A method is described for demonstrating the conducting and nervous systems of the heart. When large blocks of tissue are stained *in toto* by this technic, sections show well impregnated nerve fibers and clearly differentiated muscle fibers. Shrinkage is minimal.

Fix in 10% formalin (4% formaldehyde neutralized with ammonia) for 10 days, or longer. Place 48 hr. in: 10% formalin, 95 cc.; NH_4OH, 5 cc. Wash 48 hr. in running tap water. Wash 3 hr. in many changes of dist. water. Place 24 hr. in pyridin. Wash 4 days in many changes of dist. water. The tissue is then handled in the dark until dehydration. Put 4 days in 2% $AgNO_3$ in an incubator at 35° C. Wash 3 hr. in many changes of dist. water. Place 24 hr. in: pyrogallic acid, 4 g.; 5% formalin, 100 cc. Wash 1 hr. in dist. water. Transfer to 50% alcohol, dehydrate, clear in benzol or chloroform, and embed in paraffin.

To insure best results, the specified number of hours for fixing and staining should be rigidly adhered to. Washing should be prolonged if the reagents are not thoroly removed.—*H. D. Reed.*

DE GALANTHA, E. **A new stain for connective tissue, mucin and allied substances.** *Amer. J. Clin. Path.*, **6**, 196–7. 1936.

Orange S (E. I. duPont de Nemours & Co.) makes a good contrast stain in various combinations. The stock solution contains 1 g. orange S in 200 cc. 95% alcohol and 10 cc. glacial acetic acid. For use dilute 50 cc. with 25 cc. abs. alcohol. To stain connective tissue and mucin, treat formalin preserved sections as follows: Stain 30 min. in sat. aq. solution picric acid, 75 cc., plus glacial acetic acid, 5 cc; wash in water; stain 10 min. in alum hematoxylin (volume and concentration not specified) plus 10 drops orange S staining solution; wash in water 10 min.; stain 20 min. in alum lake carmine made up fresh each time as follows: heat 2 g. alum carmine and 0.5 g. Al Cl_3 slowly in 20 cc. dist. water, until deep red, add 100 cc. 95% alcohol and 80 cc. dist. water, filter. Wash quickly in water; stain 5 min. in a mixture of 200 cc. sat. aq. solution of picric acid and 20 cc. 1% aq. indigo carmine; wash quickly in water; dehydrate in 95% methyl alcohol, and in acetone; clear in xylene; mount with Canada balsam. The colors are: mucin, brilliant red; cartilage and hyaline substances, bluish red; fibrillar and collagenic connective tissue, grass green; smooth muscle, olive green; striated muscle, yellow. For amyloid, treat sections as follows: Harris' hematoxylin, 5 min.; steaming carbol fuchsin, 5 min.; wash in tap water; decolorize in acid alcohol (1% HCl in 70% alcohol); wash 10 min. in running tap water; stain in picro-indigo-carmine solution, 5 min. and in orange S solution, 1 min.; decolorize in abs. alcohol; wash in abs. alcohol; clear in carboyl-xylene, and xylene; mount in Canada balsam. Amyloid stains greenish gray; connective tissue, green; erythrocytes, yellow; and nuclei, reddish brown.—*V. Warbritton.*

GALL, E. A. **The technic and application of supravital staining.** *J. Lab. & Clin. Med.*, **20**, 1276–93. 1935.

A thoroly critical digest of the theory and practical use of vital staining in the diagnosis and classification of blood elements. The cell forms of normal and pathological blood are described in detail, and their characteristics are mapped out in a reference chart. All routine laboratory steps of the neutral red-Janus green technic are given. It is argued that the supravital method cannot be relied upon to the exclusion of the fixed smear technic.—*J. A. de Tomasi.*

GRAY, J. H. **Preliminary note on the mast cells of the human pituitary and of the mammalian pituitary in general.** *J. Anat.*, **69**, 153. 1935.

The pituitary was fixed by the intracardiac injection of 10% neutral formalin in normal saline during ether anesthesia. If alcoholic staining solutions were to be used, the sections were neutralized in acid or alkaline alcohol during differentiation. If the staining solutions were made up with non-alcoholic solvents, the sections were neutralized in acid or alkaline baths previous to staining.—*H. D. Reed.*

HEIDERMANNS, C. and WURMBACH, H. **Eine Methode zum histochemischen Nachweis geballten Phosphats im Gewebe.** *Zts. wiss. Mikr.*, **51**, 375–8. 1935.

So far the histochemical detection of phosphates has limited itself to the qualitative determination of the cation, mostly Ca. Now a dark red color reaction is proposed, specific for the P anion. It is based upon the property of uranyl salts to develop a ppt. with PO_4 insoluble in acetic acid, soluble in dilute mineral acids. The following schedule is suggested: from water, soak sections 1½–2 hr. in 1% uranyl sulfate; rinse in water; dip quickly in 1% HNO_3; rinse in water; treat 20 min. in 5% $K_4Fe(CN)_6$; rinse in water; dip 2–5 min. in 5% HNO_3; rinse repeatedly in water; counterstain 10 min. with thionin; dehydrate, clear and mount. Ossification centers appear a dark red-brown due to PO_4; cartilage, purple-red; connective tissue, blue; blood cells, green.—*J. A. de Tomasi.*

HIRAKO, G. **Beiträge zur wissenschaftlichen Anatomie des Nervensystems.** *Folia Anat. Jap.*, **13**,, 561–6. 1935.

The author tries out a number of methods in the study of the Purkinje cells of the cerebellum including: Nissl's, Golgi's, Bielschowsky's, Cajal's, Dogiel's vital stain, Carnoy hematoxylin, Müller-Retzius'; but by far the most satisfactory is the Weigert-Pal hematoxylin medullar stain. (Details of the methods not given.) It is not only selective for the Purkinje cells, but surpasses any of the others in developing a complete picture of even their finest outer branches.—*J. A. de Tomasi.*

KATO, K. **Monophyletic scheme of blood cell formation for clinical and laboratory reference.** *J. Lab. & Clin. Med.*, **20**, 1243–1252. 1935.

While no pretense is made of formulating a new theory of hemocytogenesis, the blood cells are once more reclassified on the basis of their clinical significance. The purpose is to systematize the nomenclature, to redefine the various cell types and to arrange them genetically in accordance to the monophyletic neounitarian theory. Staining is carried out by means of Pappenheim's panoptic combination (May Grünwald stain followed by dilute Giemsa). The scheme of classification is discussed at length and summarized in a 3-color chart commendable for its fine typographical appearance.—*J. A. de Tomasi.*

MARZA, V. D. **The formation of the hen's egg. V. Histochemistry of yolk formation. Proteins.** *Quart. J. Micr. Sci.*, **78.**, 191. 1935.

This paper contains a standardization of Unna's method for the detection of acid proteins in developing eggs. The ovaries are fixed in either 96% alcohol or in acetic sublimate and embedded in paraffin.

Three series of sections are made: a) the first is untreated and is used to detect the total acid protein; b) the second is treated, after paraffin removal, with sterile water in an incubator at 40° C. for 24 hrs.; this dissolves out the cytoses and pseudo-globulins; c) the third is treated, after paraffin removal, with sterile 2% NaCl in an incubator at 40° C. for 24 hrs.; this dissolves out the globulins as well as the cytoses leaving only the nucleoproteins.

The sections must all be the same thickness. A clamp arrangement is devised for holding slides so that all three series can be treated at once with the stain and dehydrating reagents.

The sections are stained for 20 min. in methyl-green-pyronin: methyl green (purified by shaking with chloroform), 0.15 g.; pyronin, 0.25 g.; 96% alcohol, 2.5 cc.; 0.5% carbolic acid, 100 cc. Wash rapidly in water for 1 or 2 sec. Pass thru 4 changes of 96% alcohol, 5 sec. in each. Clear in xylol and mount in Canada balsam.

Four staining intensities for pyronin can be distinguished: pale pink, light red, red, and deep red. The relative amount of acid protein can then be roughly ascertained by comparing the differently treated sections, the more acid protein the deeper red the color.—*H. D. Reed.*

MIYAMOTO, K., YAMADA, K. and SHISHIDO, S. **Dunkelfeld-Untersuchungen an überlebenden Triton-Erythrozyten. I. Vorläufige Mitteilung. Über den Einfluss der H-ionenkonzentration auf die Triton-Erythrozyten.** *Folia Anat. Jap.*, **13**, 509–12. 1935.

This darkfield investigation is part of an extended study on the influence of H-ion concentration on erythrocytes. In the blood of Triton it has been claimed that below pH 7.0 granules and rodlets appear while at a pH higher than 7.2 there is a development of a net- or mesh-like structure. The darkfield technic is applied to investigate this claim. The production of independent granules is verified and might be explained by the polarity of the erythrocytes and the incidental "gelatinization" effect of the supravital dyes, rather than by the dispersion changes of the plasma sols due to varying the H-ion concentration.—*J. A. de Tomasi.*

PASTEELS, J. and LEONARD, G. **Sur la détection du glycogène dans les coupes histologiques.** *Bull. d'Histol. Appl.*, **12**, 293–9. 1935.

This is a study of the detection of glycogen as well as of the effect of various fixatives upon trout and amphibian eggs, also various liver and muscle tissues. Altho its specificity is not the highest. Bauer's stain (formula not given) is the most reliable provided a control is run covering salivary digestion and Lugol tests. Embedding is preferably carried out by transferring the material from the fixing bath to dioxan containing a little $CaCl_2$, and after 20–30 min. passing to dioxan-paraffin, and paraffin. A large number of fixatives were studied: abs. alcohol, alcohol-formol, dextrose-formol, Sauer-Carnoy's, Zenker's, Bouin's, and Bouin-Allen's. As far as preserving glycogen is concerned, the most reliable proved to be those in aq. solution, Bouin-Allen's being the best. A still better fixative for embryonic tissues is the following: sat. picric acid soln. in dioxan about 35%), 85% by volume; formalin, 10%; glacial acetic acid, 5%. Fixation is attained in 1 hr. Other experiments show also that picric acid is preferable to various combinations of plain alcohols and formalin.—*J. A. de Tomasi.*

PICK, J. **Einige Vitalfärbungen am Frosch mit neuen fluoreszierenden Farbstoffen.** *Zts. wiss. Mikr.*, **51**, 338–51. 1935.

Generally speaking, vital and intravital stains make use of dyes whose color is perceived by transmitted illumination. There is also a group of dyes which can be seen by fluorescence thru irradiation with ultraviolet light. This group is indicated here with the generic name of "Fluorochrome" and is studied at length with respect to physical and chemical properties of its individual members. Various types of tissue from the frog serve as experimental material. It is found that dyes appearing red in transmitted light (geranin, rhodamine, eosin, magdala red, erythrosin) are not of practical value in intravital fluorescence work. The following (with their fluorescence) are more promising: primulin yellow, blue-violet; primulin, bluish violet; thiazo yellow, blue-violet; thioflavin, blue-violet; brilliant phosphin G extra, orange; rheonin A, greenish; berberin sulfate, yellowish; and styryl-chinolin, greenish. With these there should be included two plant pigments: chelidonium extract, light yellow; and extractum radicis rhei, bluish.—*J. A. de Tomasi.*

PICKWORTH, F. A. **A new method of study of the brain capillaries and its application to the regional localization of mental disorder.** *J. Anat.*, **69**, 62. 1934.

In this method the blood is stained *in situ*, thereby obviating the hazards of injecting dyes to make the blood vessels visible. Hemoglobin forms a blue-black pigment with benzidine-nitroprusside mixture in the presence of an oxidizing agent. This pigment is insoluble in organic liquids. The method can be used for other tissues.

The brain is fixed *in situ*. This is done by cutting thru the skull 1 in. above the auditory meatus and 1 in. above the eyebrow, and then, before disturbing the vault, cutting thru all tissues and brain with a fine hacksaw. The part in the vault is fixed *in situ*, and the remainder is also fixed *in situ* by attaching a 4 in. section of a 6 in. inner tube to form a basin. Fix 24 hrs. in formol hypertonic saline: 40% (commercial) formalin, 100 cc.; NaCl, 20 g.; water, 1000 cc. The brain is then cut into slices not more than 1 cm. thick and further fixed in fresh formol hypertonic saline for 2 days. Wash in water for several hours. Soak slices for 2 days in gum phenol solution: gum arabic, coarse powder, 500 g.; water, 2000 cc.; phenol, 20 g.; warm and filter thru *wool* and then by pressure thru a Leitz bacterial filter. Cut sections at 250 μ (or less) with freezing microtome. Wash in running water for 2 hrs. to remove gum and formalin. Stain sections 1 hr. in a shaking machine at 37° C. in the following benzidine-nitroprusside mixture: Sodium nitroprusside, 0.1 g.; 0.5% benzidine in 2% acetic acid, 25 cc.; dist. water, 75 cc.; dissolve the nitroprusside in 20 cc. water and add the benzidine, make up to 100 cc. by adding dist. water, and filter. The solution must be prepared directly before using. Rinse quickly in water. Place slices in weak hydrogen peroxide (2 cc. in 400 cc. dist. water) in a shaking machine at 37° C., shaking gently for 1 hr. Wash in water, dehydrate, clear in xylol, and mount in balsam.

The tissues should be clear and transparent. The blood vessels are seen as well-defined jet-black threads. If the balsam is acid-free the sections will keep well for over 6 months.—*H. D. Reed.*

ROBERTS, W. J. **Rechèrche de l'or dans les tissus animaux par la méthode du développement physique.** *Bull. d'Histol. Appl.*, **12**, 344–61. 1935.

This is a study of the sensitivity of the so-called method of "physical development" for the detection of gold in animal tissue sections. The method is based on the precipitation of Ag+ on the gold from a combination of $AgNO_3$, hydroquinone and citric acid, balanced in such a way that no Ag will separate unless Au is present. No metal should be used for fixation; formol or Bouin's fixatives are satisfactory. The technic can be used for both frozen and paraffin sections. The latter are secured to the slide with dist. water and are cleared in 3 baths of xylene. The schedule follows: Solution A: $AgNO_3$, 2 g.; 10% gum arabic, 100 cc.; prepare freshly and keep in the dark. Solution B: hydroquinone, 1 g.; 10% gum arabic, 100 cc. Hold 24 hrs. before use. Fix material in 20% formol, wash thoroly in water, gather sections in dist. water; mix 2 cc. of Sol. A with 2 cc. of Sol. B. and add 1–3 drops of 5% citric acid. Stir 30 sec. and treat the sections 5–10 min. Transfer for a few minutes to 5% $Na_2S_2O_3$. Wash thoroly and mount, with or without counterstain.—*J. A. de Tomasi.*

SCHMIDT, W. J. **Dichroitische Gold- und Silberfärbung des Aussengliedes der Sehzellen vom Frosch.** *Zts. wiss. Mikr.*, **52**, 8–23. 1935.

The idea of using heavy metal impregnation is applied to the study of retina sections. The method relies upon the precipitation of Ag as well as Au on the cellular elements and on the use of polarized light for their examination. By rotating the Nicol's prisms a double color effect is obtained which accounts for the designation "dichroic stain" given the method. Proceed as follows: Spread sections fixed in Zenker fluid (without acetic acid) upon a glass slide. Run down to water. Soak 30 min. in 0.25–0.5% $AuCl_3$ or 1% aq. $AgNO_3$ solution. Transfer to 100 cc. dist. water plus 1–2 drops hydrazine hydrate. In a fraction of a minute the reduction of the metal is completed. Wash thoroly in dist. water, run up to xylene and mount in Canada balsam. The dichroism is limited to the outer segment of cones and rods of the retina. With gold, the color produced is a dirty wine red when the cellular elements lie parallel to the vibration plane of the polarizer, and blue-gray or blue-black when they are perpendicular to it. With silver, the colors are, respectively, greenish-yellow and rust-brown or black. The balsam also acts as a reducing agent on such preparations and can be substituted for the hydrazine hydrate by simply eliminating the latter from the schedule. The reduction is ordinarily completed in a few hours, rarely requiring 1–2 days. With gold, the colors are, respectively, brilliant wine red and dark blue. All these color effects are reproduced in a trichrome plate appended to the article.—*J. A. de Tomasi.*

SOKOLANSKY, G. **Versuchsergebnisse einer Färbung von Myelin-Nervenfasern in Gefrierschnitten nach eigener Methode.** *Zts. wiss. Mikr.*, **51**, 378–83. 1935.

For the detection of myelin nerve fibers in frozen sections the following methods prove quite satisfactory: (1) For general orientation only: Fix 2–3 days in formalin; cut 20–30 μ frozen sections; soak 24–72 hr. in concentrated $K_2Cr_2O_7$ at 37–40° C.; rinse in water and stain 5–20 hr. in Kultschitsky's hematoxylin at the same temperature; transfer for 2–3 min. to the Müller's solution. Rinse in water; differentiate according to Pal (oxalic acid and 2% Na_2SO_3); abs. alcohol, carbol-xylene, and Canada balsam. (2) For finer study of details: Cut 5–8 μ frozen sections and soak 24 hr. in concentrated $K_2Cr_2O_7$ at 37–40° C.; stain 4–6 hr. in Kultschitzky's hematoxylin at 50–60° C. or 48 hr. at 40° C. Follow schedule (1) to the end. The sections ought to be kept separated in the bichromate as well as in the staining solution. At times, 10 min. in 70–80% alcohol before the bichromate prevents uneven "chromation"; after this, the yellow color must be washed away as much as possible by a water rinse.—*J. A. de Tomasi.*

THOMAS, J. A. and LAVOLLAY, J. **Une réaction histochemique du fer à la 8-hydroxyquinoléine.** *Bull. d'Histol. Appl.*, **12**, 400–2. 1935.

The reaction with 8-hydroxyquinoline, resulting in the production of a green-black compound, is considered specific for Fe+++ and can be employed for the detection and location of iron salts in animal tissues. It is not interfered with by fixatives like alcohol, trichloracetic acid, neutral formol, and Bouin's. The compound formed is soluble in the common organic solvents. The technic is as follows: Dissolve 2.5 g. 8-hydroxyquinoline (orthooxyquinoline) in 4 cc. pure acetic acid, add dist. water up to 100 cc. and filter. Place a few drops of the reagent on the sections, well fixed and washed in dist. water. After 5–15 min. drain and replace with 1 drop of conc. NH_4OH in dist. water. Wash away the resulting precipitate with dist. water. Stain the nuclei with lithium carmine. Mount in neutral dist. water or after partial drying, dehydrate in terpinol and mount in vaseline oil. Examine promptly as the intensity of the stain decreases appreciably within the first 24 hr.—*J. A. de Tomasi.*

WILLIAMS, B. G. R. **Observations on intensifying the metachromatic properties of cresylecht violet.** *J. Lab. & Clin. Med.*, **20**, 1185–7. 1935.

In the development of a new method for preparing a polychrome staining solution from cresylecht violet it is found that, contrary to expectation, alkalinization is practicable. A good dye must be used and great care should be exercised in the manipulation. The method follows: Mix cresylecht violet (National Aniline & Chem. Co.) 1 g.; dry K_2CO_3, 1 g.; formalin (U. S. P.), 5 ml.; dist. water, 95 ml. Shake at intervals for 30 min. Add 3 cc. glacial acetic acid in small amounts while agitating. Do not cork; shake at intervals for 30 min. Filter and add 5 ml. 99% isopropyl alcohol. The solution is used in surgical pathology and for rapid examination of tissues (stain the wet sections directly for about 6 sec., then wash them in tap water). The color reactions are: all nuclei and muscle cytoplasm, blue; other cytoplasm and fibrils, pink; fat cells, yellow.—*J. A. de Tomasi.*

PLANT MICROTECHNIC

KISSER, J. **Bemerkungen zum Einschluss in Glyzerin-Gelatine.** *Zts. wiss. Mikr.*, **51**, 372–4. 1935.

There are several disadvantages in the use of glycerin-gelatin as a mounting medium for plant sections. It very easily takes up impurities which then appear as comtaminants in the mount and are difficult to eliminate. Air bubbles are held fast, and the mounting medium shows a tendency to dry up and shrink. In most cases it is believed that the medium as well as the sections at the time of mounting carry too much water. A new formula for glycerin-gelatin which proved satisfactory on a pliable substrate (celluloid sheets) is the following: gelatin, 50 g.; water, 175 cc.; glycerin, 150 cc.; phenol, 2–3 g. The sections must be transferred to the mount directly from glycerin instead of from water. In the case of mounts on glass slides, the cover glass is not absolutely necessary; if used, ringing can be eliminated.—*J. A. de Tomasi.*

MICROÖRGANISMS

GRAF, W. **Untersuchungen über die Verwertbarkeit der Farbstoffreduktion zur Differentialdiagnose in der Salmonellagruppe.** *Zentbl. Bakt., I Abt. Orig.*, **135**, 377–85. 1935.

Knowledge of the theory of redox potentials is of paramount value in the choice of proper dye-stuffs to be used in bacteriological diagnostic work. Several dyes are suitable for use in nutrient media, and their color reduction is correlated to different serologic groups of the *Salmonella* genus. To make sure of uniform material Merck's broth (Standard I) at pH 7.4 is used thruout. Any sugar that may be present is taken out by yeast or *B. coli* fermentation, and semisolid media are made up with 0.1% agar. The color of the media prevents utilization of yellow and brown dyes. Concentration is a delicate item on account of the toxicity factor; for this reason it is best to add the dyes to the liquid cultures after incubation for 15–20 hr. To prevent reoxidation, a layer of paraffin oil is poured over the cultures.

Rosindulin GG (Hollborn), used in the same concentration as was used by Ishiyama for chrocein (*Zentbl. Bakt., I Abt. Orig.*, **111**, 24, 1929), is reduced within 24–36 hr. by paratyphoid B strains but not before 3 days by paratyphoid A strains. Indigo carmine, Janus green and neutral red are not satisfactory. While 50 strains of Breslau and 50 of Schottmüller could not be differentiated by 23 dyes studied, "gaslos" varieties of the two groups could be differentiated by Janus green and neutral red (but not beyond pH 7.5). Furthermore, R and S dissociation is correlated with changes in the degree of reduction of the indicators. —*J. A. de Tomasi.*

KAUFFMANN, F. **Weitere Erfahrungen mit dem Anreicherungsverfahren für Salmonellabacillen.** *Zts. Hyg. u. Infektionskr.*, **117**, 26. 1936.

As enrichment media for bacteria of the *Salmonella* group, the following were tried:

1. Modified Drigalski agar: To 1000 cc. of 2% agar in bouillon at pH 7.4–7.6 add: lactose, 15 g. 1% crystal violet, 5 cc; $Na_2S_2O_3 \cdot 5H_2O$, 1 g. Litmus solution (Kahlbaum-Schering 15%.)

2. Brilliant green agar (modification of Kristensen, Lester, and Jürgens' nutrient medium): Liebig's meat extract, 0.5%; peptone (Riedel), 1%; NaCl, 0.5%; lactose, 1.5%; phenol red solution, (0.1 *N* NaOH, 40 cc., distilled water, 460 cc.; phenol red, 1 g.) 0.5% brilliant green solution, 2.5 cc. per liter; agar, 2.5%; ph 7.0–7.2.

3. Combined "Enrichment" nutrient medium (Kauffmann):

A. Preparation of tetrathionate bouillon: Sterile bouillon, 90 cc.; sterilized $CaCO_3$, 5 g.; 5% $Na_2S_2O_3$ in dist. water, 10 cc.; iodine solution (I_2, 20 g.; KI, 25 g.; dist. water to 100 cc.), 2 cc. This mixture, unheated, is added to the following solutions:

B. 1:1000 brilliant green in dist. water, 1 cc. to 100 cc. of tetrathionate bouillon.

C. Sterile bovine bile, 5%.

The combined solutions are shaken until the $CaCO_3$ is evenly distributed, then poured into sterile test tubes to a depth of 7–8 cm. The medium is then ready for use, and may be kept for a long time before using.

Inoculation must be heavy, either by adding masses of feces directly or by adding 0.5 cc. of a fluid extract of the feces. The cultures are incubated at 37° for 16–20 hr.

By using the combined "Enrichment" nutrient medium, an increase was found in positive tests for *Salmonella* bacilli in comparison with the results obtained with modified Drigalski agar or brilliant green agar.—*H. D. Reed.*

MERLING-EISENBERG, K. B. **Internal structure of bacterial cells.** *Brit. J. Exp. Path.*, **16**, 388–93. 1935.

The author gives two methods for demonstrating the intracellular structure of bacteria. (1) A thin film of the culture is allowed to dry on a slide 0.9 mm. thick and covered with a drop of oil of high refractive index (ordinary immersion oil is sufficient in most cases) and a cover glass 0.1 mm. thick. Bacteria will remain alive for a period varying from some minutes to several weeks according to the organism employed, and may be regarded as normal during the time of examination. View the preparation with dark ground illumination. An effect especially useful in photomicrography may be brought about by the use of an E. Leitz-Wetzlar condenser (NA. 1.40) in conjunction with an apochromat objective (N.A. 1.40) stopped down to 1.37. (2) With the second method the bacteria are observed in their natural aq. media. This is possible by arranging a one-sided oblique illumination by fitting an azimuth diaphragm to the dark-ground condenser.—*M. Lois Calhoun.*

NIETO, D. **Über die Bedingungen des Spirochätennachweises in einzelnen Schnitten und ein bisher zu diesem Zweck noch nicht benutztes Prinzip.** *Zts. wiss. Mikr.*, **51**, 528–31. 1935.

The principle underlying a new spirochaete stain in individual sections (see Klin. Woch., 1775–6, 1933) is explained as follows: The sections are transferred to a mixture of $AgNO_3$ and developed as usual; but, instead of a protective colloid

(e.g., gelatin), an organic acid (acetic or citric) is used. Upon adding hydroquinone or pyrogallic acid a retardation of the development is brought about similar to that secured by means of a protective colloid.—*J. A. de Tomasi.*

STARK, C. N. and CURTIS, L. R. **Evaluation of certain media for the detection of colon organisms in milk.** *Amer. J. Pub. Health*, **26**, 354–6. 1936.

The ideal medium for detection of colon organisms in milk should permit growth and gas production by small numbers of *Escherichia-Aerobacter* organisms, and inhibit the growth of those lactose-fermenting gas-producing bacteria not belonging to this group. Pure culture tests show that crystal violet, Dominick-Lauter, gentian violet, and brilliant green bile broths are not satisfactory for this purpose, but that formate ricinoleate broth does fulfill the requirements of an ideal medium.—*M. W. Jennison.*

SWARTZ, J. H. and CONANT, F. **Direct microscopic examination of the skin. A method for the determination of the presence of fungi.** *Arch. Dermat. and Syphilol.*, **33**, 291–305. 1936.

A simple technic is presented for the microscopic examination of cutaneous scales and the determination of the presence or absence of fungi. Clear the scales in 5% solution of KOH. Transfer to a watch crystal and wash with water for 2–3 min. Heat the scales gently in a drop of the following solution on a slide: lactic acid, 1 cc.; phenol crystals, 1 g.; glycerin, 2 cc.; dist. water, 1 cc.; add 0.5% cotton blue (C_4B Poirrier). The epidermal cells become a light blue while the granular protoplasm of the fungus stains heavily. If the fungus stains slightly, try staining with 1% cotton blue in 70% alcohol instead of the above stain. Mount in clear lactophenol and press out under a cover slip. For permanent preparations clean off excess lactophenol around the cover slip and seal with Noyer's cement.

Another method of obtaining permanent preparations is to mount the stained scales in chloral hydrate and acacia: dist. water, 5 cc.; chloral hydrate, 50 g.; glycerin, 20 cc.; acacia, 30 g. The permanency has been tested only for 5 months.—*M. Lois Calhoun.*

TURNER, A. W. **Pleuro-pneumonia contagiosa Boum: The staining of the causal organism in the specific lesions.** *Austral. J. Exp. Biol. Med. Sci.*, Sept., 149. 1935.

The Nocard and Roux organism of pleuro-pneumonia in cattle can be demonstrated easily in sections of pathological tissues by a variety of staining methods providing it is suitably fixed. Formol-saline, formol-Müller, or Regaud fixatives are contra-indicated. Suitable fixatives are: the mercuric chloride group (especially Zenker), Bouin (picro-formol-acetic), abs. alcohol, and Carnoy. The recommended technic for routine purposes is fixation in Bouin's solution followed by the staining of paraffin sections with Mallory's phosphotungstic acid hematoxylin for 18–24 hr. Blot nearly dry without washing, and quickly dehydrate with abs. alcohol. Clear in xylol, mount in balsam.

The organisms stain a deep blue and appear distinctly even under the low power as thick tangled masses of the mycelial phase. The nuclei stain blue, protoplasm and muscle various shades of blue, mucin reddish yellow, cartilage deep rose red, fibrin deep blue, and erythrocytes steel blue.—*Elizabeth Bachelis.*